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INFLUENCE OF THE INGESTION OF COLOSTRUM ON THE PROTEINS OF THE BLOOD SERA OF YOUNG FOALS, KIDS, LAMBS, AND PIGS [1]

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INTRODUCTION

In a project concerned with the artificial feeding of young farm animals from birth to the end of the suckling period, at the United States Animal Husbandry Experiment Farm at Beltsville, Md., a consideration of the importance of colostrum to the young of the different species became of great interest. There is considerable evidence in the literature, principally from the laboratories of Smith (*11, 16, 17, 18, 19*),[3] indicating that colostrum, or its equivalent in the transfer of certain types of immunity, is essential for the rearing of healthy calves. It further appears, from the work of Orcutt and Howe (*14*), that in the calf at least the absorption of antibodies from the colostrum does not take place to any considerable extent except in conjunction with absorption of unchanged globulins from the colostrum ingested. The possibility that the direct absorption of globulins by the new-born animal may have some physiological significance aside from the transfer of any passive immunity should not be overlooked.

The author, therefore, has regarded the changes in the protein composition of the blood of the new-born animal following the ingestion of colostrum as a probable index of the absorption of colostrum and perhaps as some measure of the peculiar benefits available to the animal from the colostrum. This paper presents data in regard to the changes, which result from colostrum ingestion, in the protein concentration and in the distribution of protein fractions in the blood sera of young foals, kids, lambs, and pigs.

REVIEW OF LITERATURE

The function of colostrum in the early development of the young animal has been ascribed at times to its laxative properties and again to its high nutritive value, but more often to its role in the transfer of passive immunity from dam to suckling. Following the classic investigations of Ehrlich (*4*) on the transfer in mice of maternal immunity to certain phytotoxins, by means of milk, many studies

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[3] Reference is made by number (italic) to Literature Cited, p. 489.

were made on the transfer of antibodies in milk. However, the interpretation of results from these studies was confused by the fact that in some of the species used there was immunization of the young in utero, whereas in others the young were born without the immune bodies which were present in the blood of the dam.

The literature on the many aspects of the subject of transfer of maternal immunity has been comprehensively reviewed by Braun, Hofmeier, and Holzhausen (*2, pp. 1109–1146*), and reference is made in the present paper only to such investigations as are thought to have an immediate bearing on the interpretation of the results obtained in the study.

In 1922 Smith and Little (*16*) published the results of the first of a series of investigations which definitely established the importance of colostrum for the survival of the new-born calf. They showed that from 75 to 80 percent of calves deprived of colostrum failed to survive, whereas the control animals, which had received colostrum, all lived. These authors concluded (*16, p. 187*)

> that the function of the colostrum is essentially protective against miscellaneous bacteria which are harmless later when the protective functions of the calf have begun to operate and accumulate energy.

Howe (*6*) observed that the blood of the calf at birth contained neither euglobulin nor pseudoglobulin I in appreciable quantities, but that the ingestion of colostrum by the animal at any time within 2 days after birth resulted in the rapid appearance of these protein fractions in the blood. Orcutt and Howe (*14*) furnished evidence of the association of certain agglutinins with the globulin fractions in cow's colostrum and further demonstrated the simultaneous appearance of these globulin fractions and of the associated agglutinins in the blood of the new-born calf after the ingestion of the colostrum. These authors postulated a direct absorption of colostrum globulins together with the associated antibodies.

Lewis and Wells (*10*) repeated on the human infant the study which Howe made on the new-born calf. They found that in contrast to the calf, the infant has at birth a quantity of pseudoglobulin per cubic centimeter of blood comparable to that of the adult, and lacks only euglobulin. These findings were confirmed by Boyd (*1*), who further demonstrated that after the ingestion of colostrum there is a definite, although not great, increase in the quantity of serum euglobulin.

Kuttner and Ratner (*9*), in a study of the permeability of the human placenta to diphtheria antitoxin, found that the concentration of antitoxin in the blood of the umbilical cord of the infant corresponded to that in the blood of the mother. They observed no increase in antitoxin attributable to colostrum ingestion, and concluded that in the feeding of infants the significance of colostrum is not comparable to that in the feeding of calves as shown by the work of Smith and his associates (*11, 14, 16*). They considered the difference in the globulin content of the blood of infants and of calves at birth and the differences in transmission of immune substances in utero in the two species as probably arising from differences in the histological structure of the placentae of these species. They pointed out that there was apparently a greater degree of permeability for passage of antibodies and of serum globulins in species in which the placenta interposes only one layer of cells between the maternal and the fetal circulations, as in man, apes, and rodents, than in animals in which there are two or more cell

layers between the maternal and the fetal bloods. Later work on the transference of immunity in certain farm animals, reported in the following paragraphs, lends support to their conclusions that in species having histologically a more complex barrier between maternal and fetal circulations than the placenta chorialis, there is no placental transfer of immune substances.

Mason, Dalling, and Gordon (*12*), in a study of the transference of immunity to lamb dysentery in sheep, also carried out some experiments on the mare and the cow They found no placental transmission of antitoxin from dam to young in the ewe, cow, or mare, but a passage of much antitoxin into the colostrum of these animals. These authors further demonstrated the subsequent appearance of the antitoxin in the blood of the new-born young after the ingestion of colostrum containing the antitoxin.

Nelson (*13*), similarly, has shown that new-born pigs born of sows immune to vaccinia virus have no immune substances to this virus until after the ingestion of colostrum from their dams.

ANIMALS USED AND THEIR MANAGEMENT

In this study, 10 foals, 3 kids, 6 lambs, and 4 pigs were used. Four of the foals were allowed to suckle their dams, 3 foals were fed measured quantities of colostrum from their dams, and 3 foals were fed a mixture of dried cow's milk, sugar, and water, which had a total protein concentration approximating that of fresh mare's milk. One of the kids was allowed to suckle its dam, one was fed measured quantities of colostrum from its dam, and the other was fed 4 ounces of sheep colostrum for the first 3 hours and only goat's milk after the third hour. Four of the lambs were allowed to suckle their dams, and two received a mixture of dried cow's milk in water. Of the 4 pigs, all litter mates, 2 were allowed to suckle their dam and 2 were placed with a sow which had farrowed 8 days previously.

All the young animals used in this study were delivered under observation, and unless a sample of blood was obtained from the umbilical cord when it was broken, each animal was immediately removed from its dam until the first sample of blood was drawn. If the animal was to be allowed to suckle, it was then placed with its dam or with a foster dam. If it was to be hand fed, it was rubbed dry and placed in a suitable stall or pen and fed from a bottle.

EXPERIMENTAL MATERIAL AND METHODS

BLOOD SAMPLES

Protein analyses were made of blood sera obtained from the new-born animals immediately after delivery and on blood sera obtained from the same animals at intervals after the ingestion of colostrum. Corresponding data were obtained from other new-born animals which received, in place of colostrum, either milk of the same species, colostrum of another species, or a mixture of dried cow's milk and water.

The first blood sample from each of foals 1 and 2 consisted of 15 or 20 cc of blood taken from the umbilical cord as it was severed just after delivery. All other blood samples from the foals, as well as all blood samples from the kids and lambs, consisted of 8 or 10 cc of blood drawn from a jugular vein with a sterile 16-gage needle.

Blood samples from the young pigs were drawn from the left ventricle of the heart by means of heart puncture. Not more than 6 cc of blood was drawn from a pig at any one time.

Because the sample of blood required for analysis was relatively large for so small an animal as the young pig, and because the method of drawing it was necessarily somewhat drastic for use on a new-born animal, it was planned to bleed the pigs only on alternate days. Of the two pigs allowed to suckle their dams, pig 1 was to be bled the first day, pig 13 the second, pig 1 the third, etc. Unfortunately, pig 1, which was bled on the first day, was killed by the sow on the third day before the second sample of blood was obtained from it. Of the 2 pigs placed with a sow which had farrowed 8 days previously, pig 2 was to have been bled the first day, pig 4 the second, pig 2 the third, etc., but both animals died on the third day. However, the assumption that the serum of pig 13 had at birth a protein composition similar to that of its three litter mates seems warranted; therefore, pig 13 has been used as representative of the effect of colostrum ingestion.

No anticoagulant was used in the collection of the blood samples; hence, all determinations were made on serum. For the determination of the protein fractions in the blood serum, Howe's micromethod (*5*) was used. In view of his later work (*8*) on the precipitating capacity of certain salts for the globulins of blood serum, 1.00, 1.25, and 1.50 volume molar concentrations of sodium sulphate were used instead of the 14.2, 18.4, and 21.5 percent concentrations formerly employed by him for the fractionation of the globulins. Eight percent of trichloroacetic acid was used for the precipitation of total proteins. All nitrogen determinations were made by the technique of Pregl (*15*). The modified Parnas-Wagner micro-Kjeldahl apparatus described by Clark and Collip (*3*) was used.

COLOSTRUM

As a means of determining whether there is any correlation between the protein concentration of the colostrum ingested and the amount of increase in serum proteins of the young animal, protein analyses were made in most cases on the colostrum which was ingested by each animal, although for only 3 foals and 2 kids is there a record of the quantity of colostrum ingested.

Samples of colostrum from the dams suckled by young under observation consisted of from 30 to 50 cc of colostrum drawn at one milking. In every instance, the first sample of colostrum from an animal was obtained before the new-born young was permitted to suckle. The samples taken for analyses from the colostrum which was later fed by hand to the experimental animals represented the product of several milkings.

All samples of colostrum were centrifugalized to remove fat and colostric bodies, and the fat-free or skimmed colostrum, which was pipetted from under the cream, was used for the protein determinations.

Protein precipitations were made according to Howe's method for the determination of the proteins in colostrum (*7*) with the modification later suggested by him that volume molar concentrations of sodium sulphate be substituted for the volume percent concentrations originally used. This method for the determination of the proteins of

colostrum was evolved for use with cow's colostrum. However, in fractioning the proteins of the milk of the mare, the ewe, and the doe (goat) in the present study, difficulty was encountered in separating casein, pseudoglobulin I, and pseudoglobulin II by this method. For this reason pseudoglobulin I and pseudoglobulin II are recorded together as one fraction. This fraction was calculated as the difference in the nitrogen content of the filtrates after precipitation with 1.50 molar sulphate and after precipitation with 1.00 molar sodium sulphate acidified with acetic acid.

DATA AND DISCUSSION

FOALS

Table 1 shows the protein analyses of the blood sera of the foals used in this study and of the colostrum ingested. The table indicates that the euglobulin fraction of the serum proteins is either absent or is present in very small quantities in new-born foals and that the pseudoglobulin I fraction is present in only small quantities.

Results from the protein analyses of the blood sera from the 4 foals which were allowed to suckle their dams show that there was in the serum of each of foals 1, 2, and 3, after the ingestion of colostrum, an increase in euglobulin, a very marked increase in pseudoglobulin I, and likewise an increase in total globulins. Further, a comparison of data on the blood-protein fractions for foals 1 and 2 and on the concentration of globulins in the colostrum ingested by each of them suggests that the concentration of globulins in the colostrum ingested was probably a factor in the amount of increase in the serum globulins following such ingestion. In the case of foal 4, there was an increase in euglobulin, but the pseudoglobulin I fraction was not significantly changed and a decrease occurred in both pseudoglobulin II and total globulins. Foals 1 and 2 were observed to suckle their dams during the first 24 hours after birth; each of their dams apparently had a generous supply of colostrum. However, information in regard either to the quantity or to the composition of the colostrum supplied by the dams of foals 3 and 4 is lacking. These last two foals were inconveniently located for observation of frequency of suckling, and their owner objected to any handling of their dams such as would be necessary in getting colostrum samples.

Foals 5, 7, and 9 were fed measured amounts of their dams' colostrum. Protein fractions were determined on a sample from each lot or quantity of colostrum from which the individual feedings of measured amounts were taken. From these data the total quantities of colostrum globulins ingested by each foal during the first 8 hours after birth were calculated. The protein analyses on the colostrum are shown in the table; the calculated amounts of colostrum globulins ingested are given in footnotes 4, 5, and 6.

Although the data given in the table for these foals indicate that the increases which occurred in total serum globulins within 12 hours after birth were not directly proportional to the amounts of total colostrum globulins ingested, it appears that the greater the quantity of colostrum globulins ingested by these foals, the larger the accumulation of euglobulin and of pseudoglobulin I in the serum.

TABLE 1.—*Protein analyses of blood sera from foals and of the colostrum ingested by them*

[Results are expressed as grams of nitrogen per 100 cc of original sample]

Product analyzed and source	Time after delivery of foal		Globulin				Casein	Albumin	Non-protein nitrogen	Total nitrogen
			Euglobulin	Pseudoglobulin I	Pseudoglobulin II	Total				
	Days	*Hours*								
FOALS ALLOWED TO SUCKLE THEIR DAMS										
Blood serum from foal 1 [1]		0	0.010	0.046	0.065	0.121		0.465	0.056	0.642
	2		.090	.292	.172	.554		.327	.065	.946
Colostrum from dam of foal 1 [1]		1	1.259	1.510		2.769	0.748	.556	.042	4.115
		12	.288	.555		.843	.502	.323	.080	1.748
		36							.121	.642
Blood serum from foal 2 [2]		0	.000	.024	.137	.161		.458	.042	.661
	24		.066	.142	.065	.273		.396	.047	.716
Colostrum from dam of foal 2	7		.096	.087	.088	.271		.338	.053	.662
		1	.874	.928		1.802	.948	.277	.115	3.142
		12	.141	.182		.323	.376	.192	.056	.947
		24	.066	.000		.066	.290	.103	.053	.512
Blood serum from foal 3 [3]		0	.021	.042	.051	.114		.528	.032	.674
		20	.038	.135	.111	.284		.415	.039	.738
		44	.033	.133	.100	.266		.423	.045	.734
Blood serum from foal 4 [3]		0	.007	.046	.138	.191		.449	.039	.679
		24	.051	.050	.072	.173		.462	.044	.679
FOALS FED MEASURED QUANTITIES OF COLOSTRUM OF THEIR DAMS										
Blood serum from foal 5 [4]		0	0.000	0.029	0.068	0.097		0.462	0.044	0.603
		12	.058	.094	.087	.239		.391	.044	.674
Colostrum from dam of foal 5		1	.393	.351		.744	0.438	.299	.059	1.540
		6	.154	.067		.221	.098			.728
Blood serum from foal 7 [5]		0	.000	.028	.059	.087		.552	.040	.679
		12	.115	.227	.095	.437		.449	.031	.967
		24	.037	.229	.069	.335		.471	.034	.840
Colostrum from dam of foal 7		1	1.128	.964		2.092	1.050	.453	.071	3.666
		2	1.110	.864		1.974	1.056	.580	.050	3.660
		4	1.015	.458		1.473	1.495	.635	.047	3.650
		6				.878	.278	.638	.050	1.844
Blood serum from foal 9 [6]		0	.002	.054	.179	.235		.558	.034	.827
		12	.118	.108	.127	.353		.515	.034	.902
		24	.037	.204	.131	.372		.477	.031	.880
Colostrum from dam of foal 9		1	.490	.452		.942	.840	.468	.046	2.297
		3	.468	.375		.843	.498	.487	.043	1.871
FOALS RECEIVING MILK (NO COLOSTRUM) [7]										
Blood serum from foal 15		0	0.003	0.019	0.068	0.090		0.559	0.042	0.691
		12	.021	.026	.052	.099		.522	.036	.657
		33	.022	.040	.051	.113		.414	.039	.566
Blood serum from foal 16		0			.149	.230		.497	.028	.755
		12	.000			.208		.505	.022	.735
		36			.159	.182		.534	.028	.744
Blood serum from foal 17		0	.013	.024	.088	.124		.412	.035	.571
		24	.017	.007	.139	.163		.379	.036	.570

[1] Suckled vigorously within 3 hours after delivery.
[2] Suckled infrequently and listlessly during first 24 hours.
[3] Not observed between times of taking blood samples.
[4] Received, during the first 8 hours after birth, 10.3 g of colostrum globulin nitrogen.
[5] Received, during the first 8 hours after birth, 20.9 g of colostrum globulin nitrogen.
[6] Received, during the first 8 hours after birth, 17.1 g of colostrum globulin nitrogen.
[7] The milk fed was a mixture of dried cow's milk, sugar, and water, which had a total protein concentration approximating that of fresh mare's milk.

Table 1 also indicates that for foals 7 and 9 the large increases obtained for the euglobulin fraction within a few hours after the ingestion of colostrum are very transient. In each instance, at 24 hours after birth, the euglobulin fraction had dropped to a considerably lower value, which, however, was greater than that observed in

the serum of the new-born foals. On the other hand, the pseudoglobulin I fraction which, within a few hours after the ingestion of colostrum, had shown an increase comparable to that occurring in the euglobulin fraction, showed at 24 hours after birth, no such attendant decrease.

No consistency in the fluctuations in the pseudoglobulin II fraction was observed in the foals which received colostrum.

That such increases in serum globulins as occurred in foals 5, 7, and 9 do not occur when foals are fed, instead of colostrum, a product extremely poor in globulins, such as cow's milk, is indicated by the data in table 1. Foals 15, 16, and 17 were fed a mixture of dried cow's milk, sugar, and water which was comparable in total protein concentration to fresh cow's milk. In no case was there a significant increase in serum globulins. These foals succumbed within 2 weeks to one or more of the infections commonly associated with the condition known as "navel and joint ill" in foals. Foal 4 also displayed at 2 weeks of age the swollen joints, acute lameness, and marked lassitude associated with this condition. This last foal was the only one of those left with their dams in which no marked increase in pseudoglobulin I and in total globulins was observed.

KIDS

Protein analyses of sera from three kids, presented in table 2, indicate that serum euglobulin was present in very slight quantities in these animals at birth and that pseudoglobulin I was present in but little larger quantities.

TABLE 2.—*Protein analyses of blood sera from kids and of the colostrum ingested by one of them*

[Results are expressed as grams of nitrogen per 100 cc of original sample]

Product analyzed and source	Time after delivery of kid		Globulin				Casein	Albumin	Non-protein nitrogen	Total nitrogen
			Euglobulin	Pseudoglobulin I	Pseudoglobulin II	Total				
	Days	*Hours*								
Blood serum from kid 1, fed colostrum from dam [1]		1	0.006	0.093	0.177	0.276		0.299	0.023	0.598
		24	.211	.232	.197	.640		.213	.052	.905
	3		.135	.164	.254	.553		.130	.067	.750
	6		.174	.157	.185	.516		.162	.088	.766
	13		.105	.136	.165	.406		.340	.066	.812
Colostrum from dam of kid 1		1	.530	0.754		1.284	0.992	.108	.176	2.560
		16	.141	.146		.287	.539	.062	.112	1.000
		24	.048	.060		.108	.500	.077	.084	.769
Blood serum from kid 2, fed 120 cc sheep colostrum and only goat's milk after third hour [2]		0	.003	.075	.076	.154		.410	.087	.651
		24	.042	.084	.041	.167		.390	.068	.625
	3		.033	.085	.043	.161		.374	.051	.586
	6		.023	.037	.115	.175		.337	.051	.563
	20		.076	.025	.109	.210		.539	.043	.792
Blood serum from kid 3 allowed to suckle dam		0	.024	.039	.114	.177		.405	.095	.677
		24	.163	.341	.032	.536		.283	.103	.922
	3		.125	.173	.090	.388		.316	.059	.763
	6		.084	.146	.117	.347		.375	.059	.781
	20		.063	.111	.102	.276		.468	.059	.803

[1] Received during first 24 hours 15.2 g total nitrogen of which 7.7 g were from colostrum globulins.
[2] Received during first 24 hours 6.6 g total nitrogen of which 2 g were from colostrum globulins.

Twenty-four hours after the ingestion of the first goat colostrum by kids 1 and 3, the sera from these kids contained relatively large quantities of euglobulin and greatly increased quantities of pseudoglobulin I. This sharp rise in these two fractions was followed by a

gradual decrease in the concentration of both protein fractions in the blood of the animals during the time each was observed, which was 13 days in one case and 20 days in the other.

Although kid 2 received no goat colostrum, it was fed a small quantity of ewe's colostrum. The increases in euglobulin, pseudoglobulin I, and total globulins at the end of 24 hours may or may not be significant of absorption of globulin from the ewe's colostrum. The quantity of ewe's colostrum available for feeding this animal was insufficient for conclusive results in regard to the possibility of absorption of heterologous globulins as detected by the technique used. There are, however, observations in this laboratory that the new-born of one species can absorb certain proteins of another species in considerable quantities. This absorption of foreign protein has already been demonstrated by immunological reactions. Results obtained by the writer indicate that under certain conditions the absorption may be an extensive gross absorption demonstrable by chemical analysis.

LAMBS

Table 3 shows the protein analyses of the blood sera of the lambs used in this study, and of the colostrum ingested.

The blood of the four new-born lambs that were allowed to suckle their dams contained no significant quantity of euglobulin and only very small quantities of pseudoglobulin I. Within 24 hours after birth, however, large quantities of euglobulin appeared, and there was a sharp increase in the pseudoglobulin I fraction. These increases were followed by a subsequent gradual decrease in the same fractions and in total globulins during the time the lambs were studied.

Of the two lambs which were fed cow's milk instead of colostrum, lamb 39-JS was fed a 20-percent dried-milk mixture with a total protein content comparable to that of ewe's milk. Analyses of the blood serum indicated, at the end of the first 18 hours, a slight decrease in total globulin and in total nitrogen with approximately no change in the globulin-albumin ratio. Lamb 37-JH was fed a 56-percent dried-milk mixture comparable to colostrum in total protein concentration. The blood serum of this animal, after 26 hours, showed a slight increase in globulins and a small increase in the globulin-albumin ratio, which, however, was not comparable to the great increase in the globulin-albumin ratios in those animals which received colostrum.

TABLE 3.—*Protein analyses of blood sera from lambs and of colostrum ingested by them*

[Results are expressed as grams of nitrogen per 100 cc of original sample]

LAMBS ALLOWED TO SUCKLE THEIR DAMS

Product analyzed and source	Time after delivery of lamb		Globulin				Casein	Albumin	Non-protein nitrogen	Total nitrogen
			Euglobulin	Pseudoglobulin I	Pseudoglobulin II	Total				
	Days	*Hours*								
Blood serum from lamb 32-I.	-----	0	0.000	0.047	0.062	0.109	-------	0.441	0.067	0.617
	-----	24	.285	.233	.083	.601	-------	.267	.074	.941
	2	-------	-------	-------	-------	-------	-------	-------	-------	.832
	6	-------	.149	.140	.084	.373	-------	.375	.067	.815
	10	-------	.068	.125	.087	.280	-------	.447	.085	.812
Colostrum from ewe 25-A, dam of lamb 32-I.	-----	0.5	.718	0.472		1.190	1.117	.553	.107	2.967
	-----	24	.243	.172		.415	.709	.257	.109	1.550
Blood serum from lamb 39-I.	-----	0	.003	.064	.092	.159	-------	.318	.079	.556
	-----	24	.316	.218	.123	.657	-------	.261	.084	1.002
	2	-------	.201	.250	.077	.528	-------	.292	.091	.911
	4	-------	.167	.233	.110	.510	-------	.349	.051	.910
	35	-------	.085	.107	.138	.330	-------	.486	.044	.860
Colostrum from ewe 18-E, dam of lamb 39-I.	-----	0.5	1.738	.525		2.263	1.205	.282	.307	4.057
	-----	24	.092	.099		.191	.744	.155	.186	1.276
Blood serum from lamb 47-I.	-----	0	.000	.054	.152	.206	-------	.496	.040	.652
	-----	24	.322	.248	.051	.621	-------	.385	.071	1.077
	2	-------	.295	.202	.099	.596	-------	.381	.055	1.032
	4	-------	.209	.198	.071	.478	-------	.393	.075	.946
	10	-------	.146	.147	.102	.395	-------	.378	.084	.857
	38	-------	.080	.111	.208	.399	-------	.328	.062	.789
Colostrum from ewe 23-B, dam of lamb 47-I.	-----	0.5	.817	.756		1.573	1.626	.396	.038	3.633
	-----	7	.595	.199		.794	1.094	.297	.239	2.424
	-----	24	.102	.151		.253	.611	.036	.084	.984
Blood serum from lamb 53-I.	-----	0	.000	.013	.231	.244	-------	-------	-------	.741
	-----	24	.267	.220	.062	.549	-------	.363	.096	1.008
	2	-------	.190	.179	.111	.480	-------	.369	.084	.933
	5	-------	.153	.180	.112	.445	-------	-------	-------	.883
	11	-------	.130	.143	.096	.369	-------	.440	.065	.874
	18	-------	.093	.131	.103	.327	-------	.459	.070	.856
Colostrum from ewe 17-G, dam of lamb 53-I.	-----	0.5	.980	.730		1.710	1.556	.540	.256	4.062
	2	-------	.476	.003		.479	.676	.101	.136	1.392

LAMBS RECEIVING MILK (NO COLOSTRUM)

Blood serum from lamb 39-J8.[1]	-----	1	0.006	0.084	0.098	0.188	-------	0.457	0.055	0.700
	-----	18	.004	.047	.114	.165	-------	.388	.041	.594
Milk mixture fed to lamb 39-J8	-------	-------	-------	-------	-------	-------	-------	-------	-------	.860
Blood serum from lamb 37-JH[2]	-----	1	.043	.018	.086	.147	-------	.396	.054	.597
	-----	26	.052	.020	.107	.179	-------	.348	.082	.609
	-----	47	.012	.064	.123	.199	-------	.425	.032	.656
Milk mixture fed to lamb 37-JH	-------	-------	-------	-------	-------	-------	-------	-------	-------	2.400

[1] Received during first 12 hours after birth 3.42 g total nitrogen in a 20-percent mixture of dried cow's milk in water.

[2] Received during first 20 hours after birth 20.8 g total nitrogen in a 56-percent mixture of dried cow's milk in water.

PIGS

Table 4 presents the results of protein analyses of sera from four pigs. Sera from these animals at birth contained very small quantities of the globulin fractions. In pig 2, which ingested only milk and which was bled shortly after birth and again 2 days later, there was no marked increase in any of these fractions. All the animals, except pig 13, died on the third day. Within 24 hours after the birth of this pig the total globulins in the serum were about six times as great as those in the serum of new-born animals. This was due largely to the increase in euglobulin. There was a decrease in this large proportion of euglobulin during the 12 days in which the pig was observed.

TABLE 4.—*Protein analyses of blood sera from 4 pigs of the same litter, and of colostrum from the dam*

[Results are expressed in grams of nitrogen per 100 cc of original sample]

Product analyzed and source	Time after delivery of pig		Globulin				Casein	Albumin	Nonprotein nitrogen	Total nitrogen
			Euglobulin	Pseudoglobulin I	Pseudoglobulin II	Total				
Blood serum from pigs placed with sow farrowed 8 days previously:	*Days*	*Hours*								
Pig 2		0.5	0.031	0.040	0.062	0.133	.....	.....	.....	0.460
	2	.....	.035	.039	.103	.177	.....	0.191	0.121	.489
Pig 4	.....	24	.013	.080	.081	.174	.....	.162	.149	.485
Blood serum from pigs allowed to suckle dam:										
Pig 1	.....	.5	.017	.061	.060	.138	.....	.123	.188	.449
	.....	24	.607	.177	.072	.856	.....	.207	.106	1.169
Pig 13	4	.....	.295	.199	.087	.581	.....	.....	.....	.923
	12	.....	.168	.201	.097	.466	.....	.484	.052	1.002
Colostrum from dam suckled by pig 13	.....	1	1.680	0.541		2.221	1.234	.457	.242	4.154

The serum of the new-born pigs differed from the serum of the new-born of the other species in its remarkably high nonprotein nitrogen content. Results obtained from the pigs during the first 2 days after birth and before the ingestion of colostrum indicated approximately 25 percent or more of total serum nitrogen to be in the form of nonprotein nitrogen.

CONCLUSIONS

From the data presented in this paper it is concluded that the effect of colostrum ingestion on the serum proteins of young foals, kids, lambs, and pigs is similar to that observed by Howe in calves, in that after the ingestion of colostrum there is a striking rise in the concentration of total nitrogen, which rise is occasioned by large increases in the euglobulin and pseudoglobulin I fractions. The author further concludes from the work here reported that young foals, kids, lambs, and pigs which are fed homologous colostrum during the first 24 to 48 hours after birth absorb, from the colostrum ingested, euglobulin and pseudoglobulin I. It seems highly probable that absorption of immune substances takes place largely through absorption of the globulins with which they are so closely associated.

In a project concerned with the use of milk substitutes and supplements in the feeding of orphan farm animals, it appears that

any program for feeding these new-born animals should include either homologous colostrum or some substitute from which the new-born animals may absorb either the protective substances ordinarily supplied in the colostrum, or their equivalent. There is the possibility that the absorption of certain types of globulins in which the serum is deficient at birth may enable the young animal to elaborate its own antibodies.

SUMMARY

Results of studies of the protein fractions of sera from 10 foals, 3 kids, 6 lambs, and 4 pigs indicate that in each of the four species, the serum of the new-born animal is deficient in the euglobulin fraction of the serum proteins. In every instance pseudoglobulin I was present but only in very small quantities.

In those animals which received no colostrum from their respective dams there was little or no increase in serum globulins during the period of observation, with the exception of one kid whose serum contained increased globulins on the twentieth day after birth.

In the young foals, kids, lambs, and the one pig known to have received colostrum from their respective dams, there was a marked increase in total serum nitrogen within 24 hours after birth. This change resulted from increases in euglobulin and in pseudoglobulin I in the serum. There was a decrease in these fractions in the animals studied for some days after this rise.

It has been shown in young foals that the amount of absorption of euglobulin and pseudoglobulin I is related to the quantity of colostrum globulins ingested.

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A STUDY OF THE CAUSE OF VARIABILITY IN RESPONSE OF BARLEY LOOSE SMUT TO CONTROL THROUGH SEED TREATMENT WITH SURFACE DISINFECTANTS[1]

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INTRODUCTION

The present investigation was undertaken to discover the factor or factors involved in the variability of response of barley loose smut to control through treatment of the seed with surface disinfectants.

Previous to Johnson's report in 1914 (*13*) [2] it seemingly had been well established that the fungus causing loose smut (*Ustilago nuda* (Jens.) Kell. and Sw.) in barley (*Hordeum vulgare* L.) was controllable through seed disinfection only by the application of a prolonged and deeply penetrating treatment like the modified hot-water method of Freeman and Johnson (*5*). Since 1914, however, and especially in the past 10 years, it has been repeatedly demonstrated in the United States that the treatment of naturally inoculated barley seed with surface disinfectants such as formaldehyde solution and certain organic mercury solutions and dusts may completely control loose smut, reduce it, or fail to effect any appreciable reduction (*16*, *20*, *22*, *23*, *27*, *31*, *32*). In the absence of a knowledge of the factor or factors involved in the variable responses of barley loose smut to control through seed treatment with surface disinfectants, it has not seemed advisable, however, to recommend these easily applied remedies.

The modified hot-water bath has seemed the only certain method for controlling loose smut in barley through seed treatment. This treatment is so difficult to apply, however, and frequently so injurious to germination that it rarely has been used by farmers. Therefore, no practical measure for controlling loose smut of barley through seed treatment has been available. The results of studies establishing the factor responsible for the varying effectiveness of seed surface disinfectants in barley loose smut control are presented in the following pages.

REVIEW OF PREVIOUS INVESTIGATIONS

A detailed review of early investigations of the life history and control of *Ustilago nuda* has been given by Tisdale and Tapke (*30*). Since its description in 1889 (*15*) this fungus has been considered the sole pathogene causing loose smut in barley. It was shown that the loosely held spores from smutty heads are blown, washed, or carried by insects to the flowers of normal heads when the glumes are open at blooming. Except under arid conditions, as has more recently been demonstrated (*25*), these spores soon germinate, producing hyphae that grow directly into and deeply within the developing seeds. The fungus remains dormant within the seeds until they are

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[2] Reference is made by number (italic) to Literature Cited, p. 506.

sown; it revives as the seeds germinate and grows up through the young plants, causing the smutty heads. Two other items having an important bearing on the present investigation also should be noted. It was definitely established by 1914 that (1) *U. nuda* is strictly a floral-infecting smut and is incapable of producing smutted plants from mature barley seed inoculated with smut spores (*1*, *2*, *5*, *10*, *21*), and (2) the deeply embedded fungus in infected seed from inoculated flowers is not controlled by treating the seed with surface disinfectants. A prolonged and deeply penetrating seed treatment like the modified hot-water method of Freeman and Johnson (*5*) is necessary to effect control. Reports emanating from countries other than the United States apparently still are in agreement with the facts of life history and control as just cited.

Beginning with Johnson's report in 1914 (*13*), however, the findings of investigators in this country frequently have failed to parallel those of the early workers. Relative to infection, Tisdale and Tapke (*30*) in 1924 obtained from 52.5 to 100 percent of smutted plants in the varieties Alaska, Greece, Han River, Texas Winter, and Wisconsin Winter as a result of applying loose smut spores to mature seed from which the hulls had been removed. A few years later Tisdale and Griffiths (*29*) used 32 different collections of loose smut to inoculate dehulled seed of Tennessee Winter and Hannchen barley. Most of the collections produced high percentages of smutted plants in Tennessee Winter or in both of the varieties. Six collections, however, were unable to produce smutted heads in either of the varieties.

Abundant proof has been furnished in the past 10 years that, at least under certain conditions, loose smut in barley may be controlled through treatment of the seed with surface disinfectants. Tisdale et al. (*31*), using naturally inoculated seed of Cusado, Greece, Han River, Tennessee Winter, Texas Winter, and Wisconsin Winter barleys, concluded that "formaldehyde, which has been recommended for the control of covered smut of barley, controlled loose smut better than it controlled covered smut." In a later test Tisdale et al. (*32*), obtained excellent control of loose smut in Cusado, Orel, Tennessee Winter, and Wisconsin Winter by treating the naturally inoculated seed with solutions of various organic mercury compounds for 15 minutes to 1 hour. Similar results were obtained by Taylor and Zehner (*27*) in a 5-year test of Tennessee Winter and Wisconsin Winter grown from naturally inoculated seed immersed in a 0.3-percent solution of Semesan for 1 hour. In tests with dust fungicides, Leukel (*20*) obtained results indicating that control of barley loose smut through seed treatment with surface disinfectants is a function either of the host variety or of the different loose smuts associated with the different varieties. For example, through treatment of naturally inoculated seed with Ceresan or Semesan dust in 1928–29 and again in 1929–30, he obtained excellent control in Tennessee Winter and Wisconsin Winter, approximately 50 percent control in Orel, and only a negligible reduction of loose smut in Esaw. This was the state of knowledge forming the background of the present study.

METHODS OF EXPERIMENTATION

The experimental procedure, as shown later, was concerned chiefly with (1) floral and seed inoculations of barley with various collections of loose smut and (2) treatment with surface disinfectants of the seed

from hand-inoculated flowers. It was found necessary, however, to develop first a special technic in order to avoid serious injury to seed viability from the inoculation of barley flowers as well as from the application of surface disinfectants to the seed from the inoculated flowers.

Freeman and Johnson (*5*) have shown that the period during which barley flowers are susceptible to infection by *Ustilago nuda* extends from the time when the pollen is still immature until the fertilized ovary has attained approximately one-third of its mature size. In the present study the floral inoculations were made well within the limits of this period. In the early studies inoculation was performed by using forceps to open the glumes (when closed flowers were inoculated) and to place the smut on the stigma. The smut was conveniently carried in a capsule placed in a clamp on a thumb ring as shown in figure 1. The device is an adaptation of the handy pollen carrier devised by Leighty and Sando (*19*). Previous experience with this method had proved highly satisfactory in the inoculation of wheat flowers with *U. tritici* (*24*). It was found, however, in the case of barley that the method was slow and tedious because of the difficulty in opening the interlocking glumes, and that seed from flowers so inoculated is shriveled, low in vitality, and highly susceptible to injury when treated with Ceresan dust. Zeiner (*33*) also employed a floral-inoculation method involving spreading of the glumes, and reported similar results relative to low seed vitality. Undoubtedly barley is more susceptible to injury from spreading the glumes than is wheat. The inoculation of barley flowers without spreading of the glumes may be readily accomplished by clipping the upper portion of the spikelet, thereby bringing the stigma into view. This method is objectionable because the removal of the awns adversely affects kernel development, as shown by Harlan and Anthony (*7*).

A method of inoculation was evolved, however, that produced satisfactory results. It consists in dipping the pointed tips of forceps in the spore dust contained in the capsule of the thumb ring (fig. 1), then using the tips to pierce the central portion of one of the glumes and to insert inoculum on the stigma. By letting the inserted forceps expand slightly, the glume aperture is widened and the stigma is brought into view. If the tips of the forceps are spread slightly when placed in the spore dust and then pressed together, a considerable amount of inoculum is squeezed into the corrugations of the inner faces of the tips. This may be gradually deposited on the stigmas of from 5 to 10 successive flowers and results in a marked speeding of the work, reducing the time of inoculating a flower to but little more than that consumed in puncturing a glume. Seed developed from flowers thus inoculated was as plump and viable as that from uninoculated flowers. It was found, however, that the seed from inoculated flowers still was susceptible to injury when treated with Ceresan but was apparently wholly uninjured when immersed for 60 to 90 minutes in a formaldehyde solution containing 1 part of a 37- to 40-percent formaldehyde solution in 320 parts of water, followed by a thorough rinse in water. As noted later, there was need for an effective and noninjurious medium to disinfect the surface and near-surface parts of some of the seed from inoculated flowers. The modi-

fied method of inoculation and the formaldehyde seed treatment just described were adopted after the first experiment. (See table 1.)

In the use of the modified inoculation method it is distinctly advantageous to sift the smut through a 40-mesh sieve after its removal from the head. This process removes extraneous material and facilitates the handling of the inoculum. It is also an aid in determining the exact color of the mass of smut spores, the importance of which is shown later.

FIGURE 1.—Handy device for use in inoculating barley flowers with loose smut. It consists of a thumb ring with a side clamp to hold a capsule containing the inoculum. The knob on top of the ring holds the upper part of the capsule when the device is in use. (Adaptation of a pollen carrier devised by Leighty and Sando.)

In addition to speed and apparent lack of interference with normal seed development, the above-described method has the following advantages. (1) Different collections of smut may be conveniently carried to the field in individual capsules placed in envelops appropriately labeled. (In making the change from one collection of inoculum to another, only a few seconds are required to replace a capsule in the clamp of the thumb ring and to disinfect the forceps by flaming after immersion in alcohol.) (2) The insertion of inoculum only on the sticky stigma reduces to a minimum the chances of

contaminating flowers of adjacent heads with errant spores from the collection in use. (3) The small amounts of inoculum usually received from collectors may be used to advantage because none of the smut is wasted.

EXPERIMENTAL RESULTS

A test of two lots of barley in 1929 had shown that treatment of the naturally inoculated seed with Ceresan dust controlled the loose smut in a lot of Wisconsin Pedigree No. 5 from Wisconsin, but only slightly reduced it in a lot of Featherston from New York. These two varieties and the loose smuts collected from them were used in the following experiment conducted at the Arlington Experiment Farm, Rosslyn, Va., near Washington, D. C., to determine whether success or failure in obtaining control through surface disinfection of the seed might be traceable to the smuts or to the barley varieties concerned. In April 1930, flowers and seeds of the Wisconsin Pedigree No. 5 in various stages of development, as shown in table 1, were inoculated by hand with smut from each of the two varieties. Featherston was similarly inoculated. The plants bearing the flowers that were inoculated were grown from seed treated by the modified hot-water method and were smut-free and grown in a greenhouse that contained no smutted plants. The loose smut used to inoculate the flowers was fresh material collected from plants grown in another greenhouse from untreated seed of the same two lots. The seed collected from the inoculated heads was then sown in a greenhouse in the autumn of 1930. The results shown in table 1 were obtained in the spring of 1931.

TABLE 1.—*Control of loose smut in barley as influenced by the varietal source of inoculum, variety inoculated, stage of anthesis at inoculation, and treatment of the seed with Ceresan in 1930–31*

Collection no. of inoculum	Variety from which inoculum was obtained	Variety inoculated [1]	Stage of anthesis at inoculation	Seed (from inoculated flowers) untreated or treated with Ceresan	Total number of plants in 1931	Smutted plants	
						Number	Percent
100	Featherston	Featherston	Pollen green	Untreated	54	11	20.4
				Treated	28	6	21.4
		do	Pollen ripe, flowers in bloom.	Untreated	72	24	33.3
				Treated	36	12	33.3
		do	Pollen shed, ovaries ¼-½ developed.	Untreated	82	16	19.5
				Treated	54	4	7.4
		Wisconsin Pedigree No. 5.	Pollen green	Untreated	72	13	16.7
				Treated	20	3	15.0
		do	Pollen ripe, flowers in bloom.	Untreated	81	17	21.0
				Treated	18	3	16.7
		do	Pollen shed, ovaries ¼-½ developed.	Untreated	67	5	7.5
				Treated	34	1	2.9
101	Wisconsin Pedigree No. 5.	Featherston	Pollen green	Untreated	56	0	.0
				Treated	24	0	.0
		do	Pollen ripe, flowers in bloom.	Untreated	40	0	.0
				Treated	18	0	.0
		do	Pollen shed, ovaries ¼-½ developed.	Untreated	62	0	.0
				Treated	43	0	.0
		Wisconsin Pedigree No. 5.	Pollen green	Untreated	68	63	92.6
				Treated	24	0	.0
		do	Pollen ripe, flowers in bloom.	Untreated	95	89	93.7
				Treated	35	0	.0
		do	Pollen shed, ovaries ¼-½ developed.	Untreated	74	69	93.2
				Treated	44	0	.0

[1] 97 control plants of Featherston and 94 of Wisconsin Pedigree No. 5 were smut-free. They were grown from untreated seed of flowers that were not inoculated.

The data of table 1 point to the conclusion that the smut rather than the host variety determines whether or not loose smut in barley may be controlled through surface disinfection of seed from inoculated flowers. Featherston proved immune to the Wisconsin Pedigree No. 5 smut (collection no. 101). Wisconsin Pedigree No. 5, however, was highly susceptible to its own smut (collection no. 101), producing over 90 percent of smutted plants when the seed from inoculated flowers was untreated; when the seed was treated with Ceresan dust, complete control of loose smut was obtained. The different stages of anthesis at the time of floral inoculation with this smut exerted no influence on the degree of smuttedness in plants from untreated seed or on the effectiveness of seed treatment in the control of smut in plants from treated seed. The Ceresan seed treatment was ineffective on both varieties, however, when applied to seed from flowers inoculated in the bloom and prebloom stages with the Featherston smut (collection no. 100). Inoculation of the flowers with this smut shortly after blooming, i. e., when the ovaries were beginning to enlarge, resulted in a decrease in the percentage of smutted plants and an increase in the effectiveness of seed treatment. These results with the Featherston smut with respect to stage of anthesis at inoculation parallel those obtained by Freeman and Johnson (*5*) with *Ustilago nuda*.

In 1931 and 1932 experiments were conducted in the spring to determine the ability of the Featherston and Wisconsin Pedigree No. 5 smuts to produce smutted plants as a result of applying smut spores to mature barley seed. In 1931 seed of Alpha, Featherston, and Wisconsin Pedigree No. 5 barleys was inoculated with each of the two smuts collected from its respective variety (collections nos. 100 and 101). In 1932 these smuts again were used, but in most instances, as shown in table 2, they were collected from varieties other than those from which they were collected in 1931. This was done to determine any possible influence of the host variety on the pathogenicity of the causal fungus. Results obtained by Tisdale and Griffiths (*29*) had led them to suspect a relation of this kind. In 1932, seed of the 3 varieties used in 1931 and of 7 or 8 additional varieties was inoculated with each of the smut collections (nos. 100 and 101). Also in 1932, seed of Esaw barley was inoculated with the smut (collection no. 106) that naturally occurs on and usually severely attacks this variety at the Arlington farm. Previous tests by Leukel (*20*) had shown that treatment of the naturally inoculated Esaw seed with Ceresan or Semesan dusts was ineffective in smut control. In both 1931 and 1932 the seed either was obtained from plants unexposed to smut or was treated by the modified hot-water method. Following removal of the hulls, the seed was blackened with smut spores and immediately sown in moderately moistened soil in deep flats. The soil was maintained at temperatures close to 21° C. until the seedlings emerged; then the plants in the flats were placed outdoors to complete growth. The collections of inoculum were obtained from plants grown in the greenhouse and in no case were more than 1 month old when applied to the seed. All of them proved highly viable in germination tests at the time the seed was sown. Results of the test are presented in table 2.

TABLE 2.—*Incidence of loose smut in varieties of barley as a result of inoculating mature seed with 3 loose smut collections*

Collection no. of inoculum	Variety from which inoculum was obtained in—		Varieties inoculated (inoculum applied to mature seed[1])	Year of test	Total number of plants	Smutted plants	
	1931	1932				Number	Percent
100	Featherston		Alpha	1931	89	0	0.0
			Featherston	1931	87	0	.0
			Wisconsin Pedigree No. 5	1931	80	0	.0
		Featherston	Alpha	1932	78	0	.0
		do	Black Barbless	1932	54	0	.0
		do	Colsess	1932	34	0	.0
		do	Featherston	1932	104	0	.0
		do	Glabron	1932	24	0	.0
		do	Hannchen	1932	56	0	.0
		do	Nepal	1932	114	0	.0
		do	Spartan	1932	44	0	.0
		do	Trebi	1932	64	0	.0
		do	Wisconsin Pedigree No. 5	1932	122	0	.0
		Wisconsin Pedigree No. 5	Alpha	1932	76	0	.0
		do	Featherston	1932	114	0	.0
		do	Wisconsin Pedigree No. 5	1932	104	0	.0
101	Wisconsin Pedigree No. 5		Alpha	1931	117	100	85.5
			Featherston	1931	82	9	11.0
			Wisconsin Pedigree No. 5	1931	79	73	92.4
		Alpha	Alpha	1932	76	62	81.6
		do	Black Barbless	1932	26	4	15.4
		do	Colsess	1932	12	4	33.3
		do	Esaw	1932	156	6	3.8
		do	Featherston	1932	70	12	17.1
		do	Glabron	1932	6	0	.0
		do	Hannchen	1932	40	36	90.0
		do	Nepal	1932	58	0	.0
		do	Spartan	1932	12	0	.0
		do	Trebi	1932	60	60	100.0
		do	Wisconsin Pedigree No. 5	1932	112	107	95.5
106		Esaw	Esaw	1932	147	0	.0

[1] Control plants from uninoculated seed of each of the varieties used in 1931 and 1932 also were grown and were unsmutted. In each year the number of control plants of each variety was equal to or slightly greater than the number of plants of the same variety grown from seed inoculated with smut collection no. 101.

The data of table 2 give further proof of fundamental differences relative to infection between the Featherston (collection no. 100) and Wisconsin Pedigree No. 5 (collection no. 101) smuts. In no case was the former able to produce smutted plants as a result of seed inoculation. The latter, on the other hand, produced smutted plants in 11 of the 14 tests involving 11 different barley varieties, the percentage of smutted plants in one case reaching 100. The Esaw (collection no. 106) smut produced results similar to those of the Featherston smut.

It is evident from the data of table 2 that the host varieties from which the smuts were collected exerted no noticeable influence on the subsequent pathogenicity of any of the smut collections.

The failure of the Featherston (collection no. 100) and Esaw (collection no. 106) smuts to produce smutted plants as a result of seed inoculation in this experiment was not due to a lack of viability or vitality. The same collections were again used several months later (in June) at Ithaca, N. Y., in floral inoculations of most of the varieties that were seed-inoculated in the foregoing experiment. The high percentages of smut in plants grown from seed from the inoculated flowers (table 4) show that the collections were viable.

Evidence leading to definite proof that two specifically distinct loose smut fungi were responsible for the results previously noted was obtained in an experiment conducted in the spring of 1932.

Seed of eight lots of naturally inoculated Alpha barley was treated for 1½ hours in a solution of 1 part formaldehyde in 320 parts of water, then thoroughly rinsed in water and sown at the Arlington farm. At the same time untreated seed of each of the lots also was sown. The lots were collected in 1931 from fields in various parts of New York in which the incidence of loose smut was relatively high. When the plants headed, it was observed, as shown in table 3, (1) that some of the smutted heads were olivaceous brown while others were dark chocolate-brown and (2) that the formaldehyde seed treatment effectively controlled the dark-brown smut but was ineffective in the control of the olivaceous-brown smut.

TABLE 3.—*Incidence and color of loose smut heads in 8 lots of naturally inoculated Alpha barley from New York, grown from seed untreated or treated for 1½ hours in a 1 to 320 formaldehyde solution*

Seed lot no.	Seed untreated or treated with formaldehyde solution	Total number of heads	Smutted heads		Color of spore mass of loose smut heads	Smut collection no.
			Number	Percent		
1	Untreated	332	19	5.7	Dark brown	118
	Treated	284	0	.0		
2	Untreated	222	11	5.0	Dark brown	119
	Treated	253	0	.0		
3	Untreated	244	36	14.8	Dark brown	120
	Treated	267	0	.0		
4	Untreated	194	10	5.2	Dark brown	121
	Treated	240	0	.0		
5	Untreated	198	21	10.6	Dark brown	122
	Treated	266	0	.0		
6	Untreated	189	17	9.0	12 dark brown; 5 olivaceous brown	123
	Treated	240	4	1.7	Olivaceous brown	126
7	Untreated	195	16	8.2	Dark brown	124
	Treated	228	0	.0		
8	Untreated	169	9	5.3	7 dark brown; 2 olivaceous brown	125
	Treated	219	3	1.4	Olivaceous brown	127

Table 3 shows that the formaldehyde treatment completely eradicated the dark-brown smut in all seed lots. In seed lots 6 and 8, in which both smuts were present, only the olivaceous-brown smut appeared in plants from treated seed. The data in table 3, therefore, give substantial support to those in table 1 in showing that the reaction of barley to seed treatment with a surface disinfectant is a function not of the host variety but of the smut or smuts carried by the seed.

Following the collection of the data presented in table 3, an inspection of the color of the smut collections used in previous experiments (tables 1 and 2) was made. It was found that the Featherston and Esaw smuts (collections nos. 100 and 106, respectively) were olivaceous brown and that the Wisconsin Pedigree No. 5 smut (collection no. 101) was a distinctly darker brown, almost black, and that these color differences in spore mass held true regardless of the last variety from which the smut was collected.

At this time, results showing further striking differences also were obtained from studies of the Featherston and Wisconsin Pedigree No. 5 smuts and from studies of other olivaceous-brown and dark-brown collections, apart from the host plants. It was found that the olivaceous-brown-spored loose smut undoubtedly was the one referred to as *Ustilago nuda* (or one of its synonyms) in the reports of Jensen (*12*), Kellerman and Swingle (*15*), Maddox (*21*), Brefeld (*1*), Brefeld and Falck (*2*), Hecke (*8, 9*), Hori (*10*), Broili (*3*),

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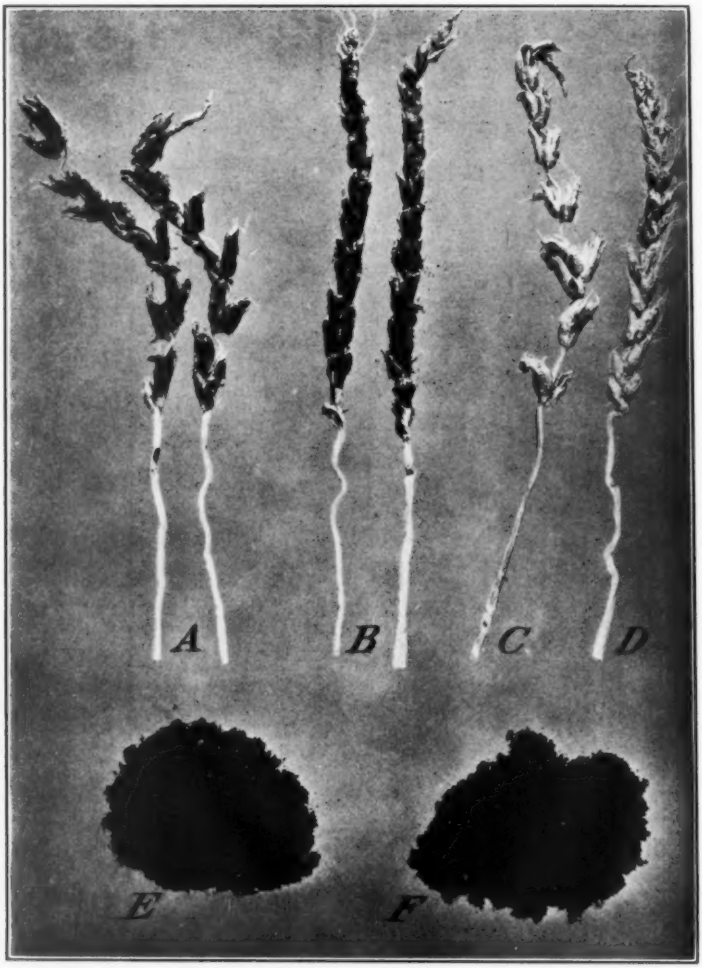

Heads of Alpha barley infected by *(A) Ustilago nuda* and *(B) U. nigra*, showing the darker color and greater compactness of the sori of the latter. Heads of Alpha barley infected by *U. nuda (C)* and *U. nigra (D)* previous to the rupture of the peridium. At this stage the color difference in heads infected by the two loose smuts of barley is not readily distinguishable. Spore masses of *U. nuda (E)* and *U. nigra (F)*, showing the darker appearance of the latter.

and Freeman and Johnson (*5*), because it conformed to the descriptions of these early workers in regard to size, color, and echinulate markings of spores, spore germination by production of a promycelium that does not bear sporidia, and ability to infect the host only through the flowers with subsequent hyphal penetration so deeply within the seed that the fungus is able to survive the action of seed-surface disinfectants. On the other hand, in addition to the distinguishing characters of the dark-brown loose smut previously noted, i. e., the darker color of its spores, its ability to produce seedling as well as floral infection, and its inability to survive the Ceresan dust and liquid formaldehyde treatments applied to seed from inoculated flowers, it was found that its chlamydospores are larger than those of *Ustilago nuda* and that on certain media they germinate by producing a promycelium bearing typically four lateral sporidia. Its chlamydospores, like those of *U. nuda*, are echinulate.

In an earlier paper the writer (*26*) proposed the name *Ustilago nigra* for this newly described darker spored loose smut. A comparison of the color of the spore masses of *U. nuda* and *U. nigra* and of the appearance of heads of Alpha barley affected with these smuts is shown in plate 1. The terms "brown" and "black" loose smuts of barley are proposed for *U. nuda* and *U. nigra*, respectively.

The foregoing studies on control of the two loose smuts of barley through surface disinfection of seed from inoculated flowers (tables 1 and 3) were restricted in regard to the number of host varieties and smut collections used. However, in 1932 and 1933, new collections were incorporated in further tests. In an experiment begun in the spring of 1932 at the Arlington farm and at Ithaca, N. Y., 22 smut collections from four States and 11 barley varieties were employed, as shown in table 4. Flowers of field-grown plants were inoculated by hand at or close to the blooming period. Seed from the inoculated flowers was collected at maturity and a part of each of the different lots was treated with formaldehyde solution (1 to 320) for 1 hour, then thoroughly rinsed in water and spread to dry for several days. Untreated and treated portions of each lot then were sown in a greenhouse at the Arlington farm in the autumn of 1932. At heading time in the spring of 1933, both olivaceous-brown (*Ustilago nuda*) and dark-brown (*U. nigra*) smut heads appeared in many of the lots. Evidently the smut collections used in the floral inoculation of such lots were mixed cultures of both species. Examination of the color and germination tests of remnant portions of the collections used in the floral inoculations confirmed this view. Soon after emergence, therefore, all of the smut heads were individually bagged. Later, when the heads were collected the olivaceous-brown and dark-brown heads from each of the various smut collections on each of the different varieties were sorted. Spores then were picked from a representative number of heads of each of the sorted lots and germinated on 2-percent potato-dextrose agar at approximately 21° C. in order to verify the species. On this medium the chlamydospores of *U. nigra* germinate and produce a promycelium bearing usually four lateral sporidia, and the chlamydospores of *U. nuda* produce a promycelium without sporidia. The species collected from smutted plants from the various lots of untreated and treated seed, as so determined, are included in the data on control presented in table 4.

TABLE 4.—*Control of Ustilago nuda and U. nigra in plants of 11 varieties of barley grown from seed from flowers inoculated by hand with 1 or more of 22 collections of loose smut from various varieties and localities in the United States*

[Seed untreated or immersed for 1 hour in a solution of 1 part of formaldehyde in 320 parts of water]

Smut collection no.	Variety and locality from which smut was collected in 1932		Varieties florally inoculated [1]	Seed (from inoculated flowers) untreated or treated with formaldehyde	Total number of plants	Smutted plants		Species of loose smut (*U. nuda* or *U. nigra*) collected from smutted heads in 1933
	Variety	Locality				Number	Percent	
	Featherston	Rosslyn, Va.	Alpha	Untreated	38	15	39.5	*U. nuda.*
	do	do	do	Treated	24	7	29.2	Do.
	Wisconsin Pedigree No. 5.	do	do	Untreated	23	13	56.5	Do.
	do	do	do	Treated	38	20	52.6	Do.
	Featherston	do	Colsess	Untreated	24	12	50.0	Do.
	do	do	do	Treated	36	19	52.8	Do.
	do	do	Featherston	Untreated	23	2	8.7	Do.
	do	do	do	Treated	46	1	2.2	Do.
	Wisconsin Pedigree No. 5.	do	do	Untreated	23	7	30.4	Do.
	do	do	do	Treated	41	18	43.9	Do.
100	Featherston	do	Glabron	Untreated	16	2	12.5	Do.
	do	do	do	Treated	36	5	13.9	Do.
	do	do	Hannchen	Untreated	24	23	95.8	Do.
	do	do	do	Treated	36	16	44.4	Do.
	do	do	Nepal	Untreated	9	4	44.4	Do.
	do	do	do	Treated	16	7	43.8	Do.
	do	do	Trebi	Untreated	21	0	.0	
	do	do	do	Treated	20	0	.0	
	do	do	Wisconsin Pedigree No. 5.	Untreated	33	16	48.5	*U. nuda.*
	do	do	do	Treated	52	6	11.5	Do.
	do	do	Wisconsin Winter.	Untreated	85	25	29.4	Do.
	do	do	do	Treated	167	23	13.8	Do.
	Wisconsin Pedigree No. 5.	do	Alpha	Untreated	29	22	75.9	*U. nigra.*
	do	do	do	Treated	41	0	.0	
	do	do	Comfort	Untreated	20	14	70.0	*U. nigra.*
	do	do	do	Treated	32	0	.0	
	do	do	Colsess	Untreated	19	9	47.4	*U. nigra.*
	do	do	do	Treated	31	0	.0	
	do	do	Featherston	Untreated	53	0	.0	
	do	do	do	Treated	73	0	.0	
	do	do	Glabron	Untreated	6	2	33.3	*U. nigra.*
101	do	do	do	Treated	8	0	.0	
	do	do	Hannchen	Untreated	13	11	84.6	*U. nigra.*
	do	do	do	Treated	32	0	.0	
	do	do	Trebi	Untreated	40	22	55.0	*U. nigra.*
	do	do	do	Treated	52	0	.0	
	do	do	Wisconsin No. 38.	Untreated	25	0	.0	
	do	do	do	Treated	41	0	.0	
	do	do	Wisconsin Winter.	Untreated	74	41	55.4	*U. nigra.*
	do	do	do	Treated	168	0	.0	
	Esaw	do	Alpha	Untreated	12	3	25.0	*U. nuda.*
	do	do	do	Treated	35	4	11.4	
106	do	do	Wisconsin Winter.	Untreated	54	25	46.3	*U. nuda.*
	do	do	do	Treated	100	28	28.0	
107	Wisconsin Winter.	do	do	Untreated	69	36	52.2	*U. nigra.*
	do	do	do	Treated	146	0	.0	
108	Beardless No. 6	do	Alpha	Untreated	28	2	7.1	*U. nuda.*
	do	do	do	Treated	58	4	6.9	Do.
	Alpha	do	do	Untreated	26	20	76.9	Both species.
	do	do	do	Treated	28	8	28.6	*U. nuda.*
109	do	do	Wisconsin Winter.	Untreated	80	41	51.3	Both species.
	do	do	do	Treated	168	6	3.6	*U. nuda.*
110	Rowan	Raleigh, N. C.	Alpha	Untreated	10	9	90.0	*U. nigra.*
	do	do	do	Treated	13	0	.0	
113	do	Middleburg, Va.	do	Untreated	13	12	92.3	Both species.
	do	do	do	Treated	18	2	11.1	*U. nuda.*
14	do	Hillsboro, Va.	do	Untreated	13	12	92.3	Both species.
	do	do	do	Treated	20	1	5.0	*U. nuda.*

See footnote at end of table.

TABLE 4.—*Control of Ustilago nuda and U. nigra in plants of 11 varieties of barley grown from seed from flowers inoculated by hand with 1 or more of 22 collection of loose smut from various varieties and localities in the United States*—Continued

Smut collection no.	Variety and locality from which smut was collected in 1932		Varieties florally inoculated [1]	Seed (from inoculated flowers) untreated or treated with formaldehyde	Total number of plants	Smutted plants		Species of loose smut (*U. nuda* or *U. nigra*) collected from smutted heads in 1933
	Variety	Locality				Number	Percent	
115	Colsess	Rosslyn, Va.	Alpha	Untreated	18	6	33.3	Both species.
	do	do	do	Treated	27	6	22.2	*U. nuda.*
116	do	do	Colsess	Untreated	20	7	35.0	Both species.
	do	do	do	Treated	40	7	17.5	*U. nuda.*
	do	Watson, Va.	Alpha	Untreated	15	15	100.0	Both species.
	do	do	do	Treated	21	1	4.8	*U. nuda.*
117	Black Barbless	Rosslyn, Va.	do	Untreated	7	7	100.0	Both species.
	do	do	do	Treated	22	1	4.5	*U. nuda.*
	do	do	Comfort	Untreated	16	14	87.5	Both species.
	do	do	do	Treated	30	1	3.3	*U. nuda.*
	do	do	Wisconsin Winter.	Untreated	81	21	25.9	Both species.
	do	do	do	Treated	168	28	16.7	*U. nuda.*
119	Alpha	do	Alpha	Untreated	13	13	100.0	*U. nigra.*
	do	do	do	Treated	19	0	.0	
	do	do	do	Untreated	42	42	100.0	*U. nigra.*
120	do	do	do	Treated	42	0	.0	
	do	do	Comfort	Untreated	31	21	67.7	*U. nigra.*
	do	do	do	Treated	43	0	.0	
126	do	do	Alpha	Untreated	37	16	43.2	*U. nuda.*
	do	do	do	Treated	37	15	40.5	Do.
	Spartan	do	do	Untreated	20	7	35.0	Both species.
129	do	do	do	Treated	31	9	29.0	*U. nuda.*
	do	do	Hannchen	Untreated	46	31	67.4	Both species.
	do	do	do	Treated	47	16	34.0	*U. nuda.*
	do	Buchanan County, Mo.	Alpha	Untreated	13	8	61.5	*U. nigra.*
	do	do	do	Treated	19	0	.0	
133	do	do	Comfort	Untreated	12	11	91.7	*U. nigra.*
	do	do	do	Treated	31	0	.0	
	do	do	Wisconsin No. 38.	Untreated	66	8	12.1	*U. nigra.*
	do	do	do	Treated	57	0	.0	
134	do	Ithaca, N. Y.	Alpha	Untreated	6	2	33.3	*U. nuda.*
	do	do	do	Treated	18	4	22.2	Do.
	Alpha	do	do	Untreated	16	14	87.5	Both species.
135	do	do	do	Treated	25	7	28.0	*U. nuda.*
	do	do	Comfort	Untreated	34	20	58.8	Both species
	do	do	do	Treated	45	4	8.9	*U. nuda.*
136	do	Marion, N. Y.	Alpha	Untreated	3	3	100.0	Both species.
	do	do	do	Treated	12	2	16.7	*U. nuda.*
137	Wisconsin No. 38.	Ithaca, N. Y.	do	Untreated	7	6	85.7	Both species.
	do	do	do	Treated	18	5	27.8	*U. nuda.*
138	do	do	Wisconsin No. 38.	Untreated	12	7	58.3	Both species.
	do	do	do	Treated	21	6	28.6	*U. nuda.*

[1] Control plants also were grown, from seed of heads not artificially inoculated, of each of the varieties used in the floral inoculation test. 100 seeds of each variety were sown and from 83 to 100 plants matured. A trace of loose smut (*U. nigra*), resulting from natural infection, appeared in Wisconsin Winter. Control plants of the other varieties were smut-free.

The data in table 4 confirm and amplify those in tables 1 and 3 in showing that the treatment of seed from inoculated flowers with certain surface disinfectants is highly effective in the control of *Ustilago nigra* but relatively ineffective against *U. nuda*. In lots in which both species appeared in plants from untreated seed, *U. nigra* was completely controlled as a result of seed treatment and only *U. nuda* appeared in the plants from treated seed. Attention is directed particularly to the action of the formaldehyde seed treatment on control of the loose smuts in Wisconsin Winter in this experiment.

Naturally inoculated seed of this variety from the agronomy plots at the Arlington farm was used by Tisdale et al. (*31*, *32*), Taylor and Zehner (*27*), and Leukel (*20*) in a series of seed-treatment studies, conducted in 9 of the 10 years from 1921 to 1930, in which treatment of the naturally inoculated seed with formaldehyde and various organic mercury solutions and several chemical dusts invariably resulted in effective control of the loose smut. In the writer's test (table 4) similar results were obtained when Wisconsin Winter was florally inoculated with the smut that naturally occurs on it at the Arlington farm (collection no. 107, table 4). Floral inoculations with *U. nigra* (collection no. 101) gave similar results. However, different results were obtained when the inoculations were made with collections that contained *U. nuda* alone (collections nos. 100 and 106) or in mixture with *U. nigra* (collections nos. 109 and 117). It is therefore evident from this and the other examples in table 4 that treatment of barley seed from inoculated flowers with surface disinfectants may eradicate loose smut, partially control it, or prove ineffective in control, depending upon the loose smut or smuts carried by the seed and irrespective of the barley variety concerned. Doubtless to this fact may be attributed the conflicting reports cited under Review of Previous Investigations or regarding the control of loose smut in barley through treatment of the seed with surface disinfectants.

Of the varieties florally inoculated with *Ustilago nuda*, seed treatment effected partial control in some cases, as shown in table 4. This may have been because some of the florets were inoculated after the fertilized ovaries had begun to enlarge. The very rapid rate of development of barley kernels following fertilization has been shown by Harlan (*6*). Apparently the depth of penetration of the infection hyphae into the young seed tissues is restricted under these conditions and the hyphae are consequently less protected from the action of surface or subsurface disinfectants applied to the seed. As noted in table 1, partial control of *U. nuda* (collection no. 100) also was obtained by inoculating flowers of Featherston and Wisconsin Pedigree No. 5 when the ovaries were one-fourth to one-third developed and the seed was treated with Ceresan dust. Freeman and Johnson (*5*) have reported similar results.

With reference to varietal resistance and susceptibility, table 4 shows that Featherston was resistant to *Ustilago nuda* (collection no. 100) and immune to *U. nigra* (collection no. 101). In earlier experiments, however, this variety proved fairly susceptible to *U. nuda* (collection no. 100, table 1) when grown from seed from inoculated flowers, and only moderately resistant to *U. nigra* (collection no. 101, table 2) when grown from seed inoculated at the time of sowing. In these experiments, therefore, seed inoculation with *U. nigra* proved more effective than floral inoculation in the production of smutted plants in Featherston. Trebi proved immune to *U. nuda* (collection no. 100) but highly susceptible to *U. nigra* (collection no. 101). None of the other varieties was markedly resistant to either of the loose smuts. Alpha and Hannchen were highly susceptible to both *U. nuda* and *U. nigra*.

The foregoing comparative studies of *Ustilago nuda* and *U. nigra* relative to the production of smutted plants as a result of seed inoculation and seedling infection have been limited to three smut collections (nos. 100, 101, and 106), as shown in table 2. The results of

further seed-inoculation studies, made in the spring of 1933 at the Arlington farm, are presented in table 5. Five loose smut collections (nos. 110, 120, 126, 133, and 134) not used in the previous seed-inoculation test were employed. They were collected from plants grown in a greenhouse and used in the preceding experiment. The seed of the varieties inoculated was first treated with hot water (modified method), left to dry during several days, blackened with smut, and then immediately sown in deep flats in moderately moist soil maintained close to 21° C. until the seedlings had fully emerged. Then the plants in flats were placed outdoors to complete growth. A germination test of each of the smut collections at sowing time showed that all were highly viable. Results from a seed inoculation of Esaw with smut collection no. 101 (*U. nigra*) also are included in table 5 because they show an influence of the host from which the smut is collected on the pathogenicity of the inoculum under certain conditions.

TABLE 5.—*Incidence of loose smut in varieties of barley as a result of inoculating mature seed with 2 collections of Ustilago nuda and 4 of U. nigra*

Collection no. of inoculum	*Ustilago* species of loose smut	Variety from which smut was collected in—		Variety inoculated (inoculum applied to mature seed)[1]	Total number of plants	Smutted plants	
		1932	1933			Number	Percent
101	*U. nigra*	Alpha	Esaw	Esaw	93	36	38.7
		do	Trebi	do	88	4	4.5
110	do	Rowan	Alpha	Alpha	38	4	10.5
		do	do	Hannchen	46	0	.0
		do	do	Trebi	42	0	.0
120	do	Alpha	do	Alpha	36	14	38.9
		do	do	Hannchen	42	12	28.6
		do	do	Trebi	42	13	31.0
133	do		do	Alpha	30	4	13.3
			do	Hannchen	46	10	21.7
			do	Trebi	28	2	7.1
126	*U. nuda*	Alpha	do	Alpha	70	0	.0
		do	do	Hannchen	91	0	.0
		do	do	Trebi	86	0	.0
134	do	do	do	Alpha	67	0	.0
		do	do	Hannchen	92	0	.0
		do	do	Trebi	83	0	.0

[1] From 78 to 95 control plants from uninoculated seed of each of the varieties also were grown, and were smut-free.

The data of table 5 show that all of the *Ustilago nigra* collections (nos. 101, 110, 120, and 133) were able to produce smutted plants as a result of seed inoculation and seedling infection. The *U. nuda* collections (nos. 126 and 134) were wholly unable, however, to produce smutted plants through seed inoculation despite the fact that, as previously shown (table 4), at least in the variety Alpha, they were able to produce high percentages of smut in plants grown from seed from inoculated flowers. These results confirm those obtained in the previous seed-inoculation test (table 2) with *U. nuda* (collections nos. 100 and 106) and *U. nigra* (collection no. 101).

The data of table 5 also show that when Esaw was seed-inoculated with smut collection 101 (*Ustilago nigra*) from Esaw a considerably higher percentage of infected plants was obtained than when it was inoculated with smut collection 101 (*U. nigra*) from Trebi. The Esaw and Trebi plants that furnished the smut in 1933 were grown from seed from flowers inoculated with the same smut collection

(*U. nigra* no. 101) from Alpha in 1932. Tisdale and Griffiths (*29*) obtained similar results with other barleys and loose smut collections and suggested that the host variety might affect the pathogenicity of the inoculum through a definite influence on either (1) the parasitism of a single physiologic form or (2) alteration in the relative content of two or more component physiologic forms.

USTILAGO NIGRA IN RELATION TO PREVIOUS INVESTIGATIONS

Knowledge of the occurrence of *Ustilago nigra* in the United States appears to explain the conflicting reports relative to barley-seed treatment with surface disinfectants as previously noted, and also seems to facilitate an interpretation of the hitherto unexplained results of the previous investigations of Tisdale and Tapke (*30*) and Taylor and Zehner (*28*).

In 1924 Tisdale and Tapke (*30*), unaware of the existence of *Ustilago nigra*, reported their discovery of the "infection of barley by *Ustilago nuda* through seed inoculation." In the present investigation, however, as shown in tables 2 and 5, the results of the writer have confirmed those of previous investigators (*2, 3, 5, 10, 16*) in showing that *U. nuda* is wholly incapable of producing smutted plants as a result of the inoculation of mature barley seed with smut spores. That the loose smut used by Tisdale and Tapke (*30*) was *U. nigra* seems likely in view of the fact that their inoculum was collected from Tennessee Winter grown at the Arlington farm in 1922. Examination by the writer of collections of the loose smut naturally occurring on this variety at that place for the past 5 years has shown that only *U. nigra* was present. Furthermore, in a series of seed-treatment studies conducted by Tisdale et al. (*31, 32*), Taylor and Zehner (*27*), and Leukel (*20*), in 9 of the 10 years from 1921 to 1930, naturally inoculated seed of Tennessee Winter from the Arlington farm was among the varieties employed, and its loose smut invariably proved amenable to control through treatment of the naturally inoculated seed with surface disinfectants. As shown in tables 1, 3, and 4, *U. nuda* is not controlled by this measure.

That Tisdale and Tapke (*30*) were working with *Ustilago nuda* and not *U. nigra* might be assumed from the fact that in a cytologic examination of seedlings grown from inoculated seed on moist paper, at approximately 21° C., it was clearly shown that the promycelia of the germinating chlamydospores directly penetrated the tissue of the coleoptile and young leaves. In no instance did they bear the sporidia characteristic of *U. nigra* when germinated on potato-dextrose agar. The production or nonproduction of sporidia by the promycelium of the germinating chlamydospore cannot be used, however, as a diagnostic character of the species under all conditions. Promycelia of *U. nigra* may be made to produce sporidia abundantly or sparsely or to branch freely without production of any sporidia by using different substrata on which to germinate the spores. Jones (*14*) and Hüttig (*11*) have obtained similar results with other cereal-smut fungi through the use of low or high temperatures, and Kolk (*17*) also has observed the direct penetration of an oat seedling by the germ tube of a chlamydospore of the loose smut fungus of oats (*U. avenae* (Pers.) Jens.), which normally produces sporidia in culture.

In 1931 Taylor and Zehner (*28*) reported their results on the effect of depth of seeding on the occurrence of covered and loose smuts in

winter barley at the Arlington farm. They found that both loose and covered smuts were materially increased in the Wisconsin Winter variety when the naturally inoculated seed was sown 3 inches deep, as compared with their occurrence when the seed was sown one-half inch deep. However, the varieties Esaw and Beardless No. 6, also grown from naturally inoculated seed, "showed no significant difference in loose-smut infection from variation in seeding depth." Owing to resistance, the two last-named varieties yielded no data on covered smut. In 1932, the writer collected loose smut at the Arlington farm from each of the same three barleys previously used by Taylor and Zehner. These smuts were used in 1932 and 1933 in the floral-inoculation seed-treatment experiment summarized in table 4. Conclusive evidence was obtained that the smut collected from Wisconsin Winter was *Ustilago nigra* and the smuts from Esaw and Beardless No. 6 were *U. nuda*. Further studies on the color of the sifted spore masses of these smuts and their mode of germination on 2-percent potato-dextrose agar confirmed their identity as noted. It seems likely, therefore, that Taylor and Zehner also were dealing with *U. nigra* on Wisconsin Winter and *U. nuda* on Esaw and Beardless No. 6. On this assumption the results of Taylor and Zehner might be explained as follows: Hecke (*9*), Broili (*3*), Broili and Schikkorra (*4*), and Lang (*18*) have shown that following floral inoculation *U. nuda* penetrates deeply within the developing seed and has thoroughly ramified the tissues of the embryo and endosperm by the time the seed is ripe. Therefore, infection, the critical point in the life of the parasite, has been well established before sowing, and subsequent influences brought about by variation in depth of seeding would not apply to this phase. In the case of *U. nigra* on Wisconsin Winter, however, the growth of the fungus following floral infection appears to be confined to the superficial layers of the seed, as evidenced by the fact that surface disinfection of the seed with formaldehyde solution effects complete control (table 4). With *U. nigra*, therefore, infection of the seedling remains to be accomplished when the seed is ripe, and variation in environment or in seedling susceptibility brought about by different depths of seeding might reasonably be expected to exert an influence on infection and on the degree of smuttedness in the plants.

CONCLUSIONS

Knowledge of the occurrence of *Ustilago nigra* in the United States seems to explain the variable results in control hitherto obtained from the application of surface disinfectants to barley seed harboring loose smut. It should also effect a reduction in loss from loose smut through a wider use of the easily applied surface disinfectants where it is known that the *nigra* species is the causal organism. Knowledge of the occurrence of *U. nigra* doubtless also should facilitate studies on (1) the genetic relation of the barley smuts, (2) the occurrence of physiologic forms in the barley loose smuts, and (3) breeding for resistance to these smuts.

SUMMARY

During the past 20 years it has been frequently reported in the United States that loose smut in barley may be reduced or eliminated through treatment of the harboring seed with certain easily applied surface disinfectants. This is at variance with the reports of all of

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the earlier and some of the recent investigators, who have found that it is necessary to apply the difficult modified hot-water treatment or its equivalent in order to control loose smut in barley through seed treatment.

The present investigation has shown that in addition to the well-known loose smut of barley caused by *Ustilago nuda* (Jens.) Kell. and Sw. a second barley loose smut caused by *U. nigra* Tapke is widely distributed and causes considerable loss in the United States. The latter species is controllable through treatment of the seed with certain surface disinfectants, and doubtless has been the factor responsible for the conflicting reports noted above.

Ustilago nigra resembles *U. nuda* in the appearance of smutted heads, in the emergence of smutted heads and dissemination of spores during the heading and flowering period of unsmutted plants, and in the inoculation of the host through the flowers.

Ustilago nigra may be distinguished from *U. nuda* by the following characteristics: (1) The color of the spore mass of *U. nigra* on fully emerged heads is dark chocolate-brown, whereas that of *U. nuda* is olivaceous brown. (2) In seed from flowers inoculated at blooming, *U. nigra* is amenable to control through seed treatment with certain surface disinfectants. The modified hot-water treatment or its equivalent is necessary to control *U. nuda*. (3) On 2-percent potato-dextrose agar at 70° F., the spores of *U. nigra* germinate by producing a promycelium bearing typically four lateral sporidia. Under these conditions the spores of *U. nuda* produce a promycelium that does not bear sporidia. (4) *U. nigra* is able to produce smutted plants through infection of the seedling as a result of inoculating mature barley seed, as well as through infection of the flowers. *U. nuda* is able to produce infection only through the flowers.

Some of the most important varieties of barley in the United States are highly susceptible to *Ustilago nigra*.

To facilitate distinction, the terms "brown" and "black" loose smut of barley are proposed for *Ustilago nuda* and *U. nigra*, respectively.

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THE SUGAR BEET NEMATODE AND OTHER INDIGENOUS NEMIC PARASITES OF SHADSCALE[1]

By GERALD THORNE

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INTRODUCTION

The original sources of nemic parasites are generally unknown. It is, therefore, of considerable interest to find the sugar beet nematode (*Heterodera schachtii* Schmidt, 1871), *Anguillulina aberrans* n. sp., and *Neotylenchus latus* n. sp. under conditions that apparently leave no doubt as to their native origin. On May 25, 1927, *H. schachtii* and *A. aberrans* were found infesting the roots of shadscale, *Atriplex confertifolia* (Torr. and Frem.) S. Wats., collected in the desert foothills west of Utah Lake, Utah. Collections made in 1933 extended the desert range of these two nemas into Cedar Valley, several miles west of the first collection, and to the hills west of Richfield, about 95 miles south. *N. latus* also was found in the material collected west of Richfield and in a single collection made west of Utah Lake.

THE SUGAR BEET NEMATODE

The fact that the sugar beet nematode (*Heterodera schachtii*) is indigenous to the desert is not surprising when one considers the morphology of the brown cyst stage. This remarkable adaptation to adverse circumstances doubtless originated under ecological conditions similar to those of its present native habitat. That this species is indigenous to both Europe and North America is not surprising, for many nemas are cosmopolitan. The Division of Nematology collection at Salt Lake City, Utah, contains over 100 species which are native to virgin soils of the United States and which also have been reported from Europe and other parts of the world.

Previous reports have indicated that *Heterodera schachtii* is a species indigenous to the United States. Maxson[2] found specimens on *Rumex* sp. in the Salinas Valley, Calif., in 1913, which he believed could not have come from introduced nemas. Steiner[3] discovered *Polygonum pensylvanicum* L. and *P. punctatum* Ell. infested with *H. schachtii* at Broad Run, near Leesburg, Va., under circumstances that might indicate native colonies in that locality.

These indigenous colonies of sugar beet nematodes explain the probable source of many infestations in the beet fields of Utah. Originally a considerable portion of the present beet land was "shadscale flats", comparatively level areas bearing almost pure stands of shadscale which were destroyed by cultivation. There is a possibility that the nemas lived on some desert plants that survived

[1] Received for publication June 26, 1935; issued December 1935.

[2] Oral communication in 1934 from A. C. Maxson, Great Western Sugar Co., Denver, Colo.

[3] STEINER, G. THE FINDING OF HETERODERA SCHACHTII, THE SUGAR BEET NEMA, ON POLYGONUM IN VIRGINIA. U. S. Dept. Agr., Bur. Plant Indus. Plant Disease Reptr. 15: 145. 1931. [Mimeographed.]

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cultivation, or perhaps they transferred from shadscale to new hosts, such as species of *Brassica*, *Rumex*, and *Atriplex*, on which they lived until sugar beets were introduced many years later.

Native colonies doubtless do not account for all nemic infestations now present in beet-growing areas. Transmission of nemas in beet seed from Europe has been known to occur [4] and probably is responsible for many infestations.

Comparisons of specimens from the desert with those from beet fields show only slight variations in form and size, the principal difference being in the length of the male tails (fig. 1, *B*), which in the

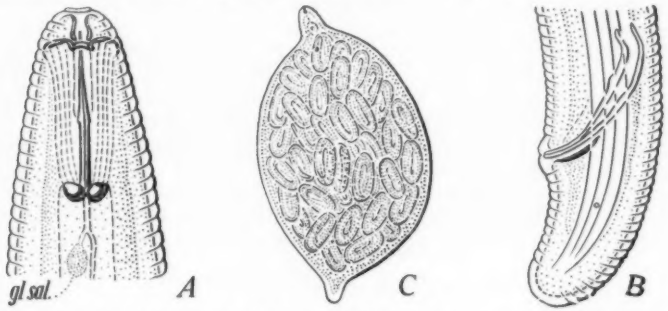

FIGURE 1.—*Heterodera schachtii*: *A*, Male head; *gl sal*, salivary gland, × 1,300. *B*, Male tail, × 1,000. *C*, Cyst, × 60.

desert form are 2 to 3 times as long as those of nemas found in beet fields. Long-tailed specimens occasionally are found living on beets in Utah, and Cobb [5] figures a similar form from Colorado.

EFFECT ON HOST

Infestations generally are limited to small groups of shadscale bushes, and there may be several rods, or even a mile, between infestations. However, the locating of infested plants is largely a matter of chance, for no visible injury or appreciable damage is shown by *Brassica*, *Atriplex*, and other common weed hosts. Infested bushes frequently are found along a watercourse and about its drainage basin.

ANGUILLULINA ABERRANS N. SP.

Anguillulina aberrans, n. sp., exhibits characters that indicate a relationship to both *Heterodera* and *Tylenchulus*. The male head of *A. aberrans* (fig. 2, *C*) is indistinguishable from that of *H. schachtii* (fig. 1, *A*). The distorted, variable-formed females of *A. aberrans* are suggestive of the females of the root knot nematode, *H. marioni* (Cornu, 1879) Goodey, 1932, from hard, woody tissues in which the body is unable to assume its typical pyriform shape. The chief difference lies in the appearance of the female terminus, which retains its identity in *A. aberrans* but completely loses it in *H. marioni*.

[4] THORNE, G. CONTROL OF THE SUGAR-BEET NEMATODE BY CROP ROTATION. U. S. Dept. Agr. Farmers' Bull. 1514, 21 pp., illus. 1926. See p. 6.

[5] COBB, N. A. ESTIMATING THE NEMA POPULATION OF SOIL, WITH SPECIAL REFERENCE TO THE SUGAR-BEET AND ROOT-GALL NEMAS, HETERODERA SCHACHTII SCHMIDT AND HETERODERA RADICICOLA (GREEF) MÜLLER, AND WITH A DESCRIPTION OF TYLENCHOLAIMUS AEQUALIS N. SP. U. S. Dept. Agr., Bur. Plant Indus. Agr. Technol. Circ. 1, 48 pp., illus. 1918. See p. 25, fig. 17.

The females of *Anguillulina aberrans* are in many respects similar in development and appearance to the females of *Tylenchulus semipenetrans* Cobb [6], but the former species differs from *Tylenchulus* in possessing the following characters: Anus functional, excretory pore near base of esophagus, male spear normal, and bursa present.

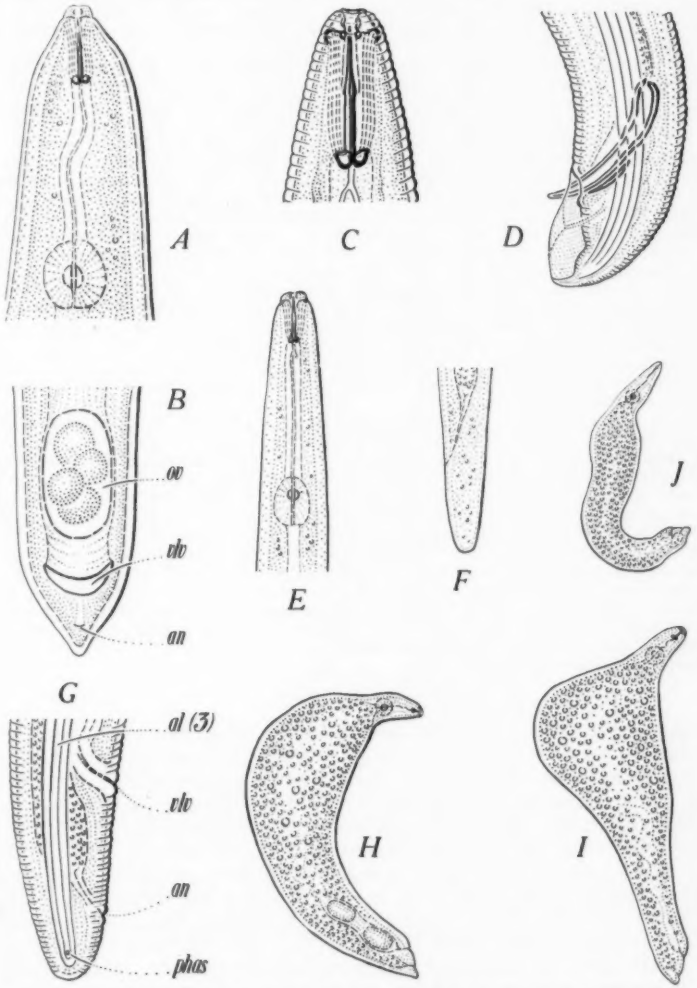

FIGURE 2.—*Anguillulina aberrans*. *A*, Anterior end of adult female, × 500. *B*, Posterior end of adult female: *ov*, segmenting egg; *vlv*, vulva; *an*, anus, × 250. *C*, Male head, × 1,000. *D*, Posterior portion of male, × 1,000. *E*, Anterior end of larva, × 500. *F*, Posterior end of larva, × 500. *G*, Posterior end of young female: *al*, wings (three); *vlv*, vulva; *an*, anus; *phas*, phasmid, × 500. *H*, *I*, *J*, Variations in size and form of adult females, × 60.

[6] COBB, N. A. NOTES ON MONONCHUS AND TYLENCHULUS. Jour. Wash. Acad. Sci. 3: 287–288, illus. 1913.

MORPHOLOGY

Eggs.—Average size 42μ×80μ. Found within the roots. Deposited before segmentation in masses covered by gelatinous secretion from the female. Senile females frequently contain a few undeposited eggs in which segmentation and hatching may occur within the body (fig. 2, *B*).

Larvae (fig. 2, *E*, *F*).—Length 0.4 to 0.48 mm. Width 20μ to 24μ. Spear length 14μ. Esophagus occupying about one-fifth body length. Intestine densely granular, granules varying in size from very minute to one-fourth body width. Tail length generally about twice anal body diameter, tapering but little to broad, rounded terminus.

Male (fig. 2, *C*, *D*).—Length 0.7 to 0.9 mm. Width 25μ to 33μ. Body almost cylindrical between esophagus and anus, tapering to lip region, which is not set off in any manner. Tail slightly longer than anal body diameter, completely enveloped by bursa. Wings three, marked by four refractive lines, the area occupying one-fourth of body width. Six obscure, low, flat lips grouped close about the entrance to vestibule; from lateral view they frequently give appearance of very thin disk. Cuticular framework of lips massive, six-parted. Spear 21μ to 25μ long with large basal knobs. Median esophageal bulb about half as wide as neck. Terminal bulb obscure, assimilated by intestine. Excretory pore distinct, located 1 or 2 body widths behind median bulb. Testis single, outstretched. Spicula slightly arcuate, tapering in distal two-thirds. Gubernaculum thin, flat, slightly arcuate. Bursa rising close in front of anus, enveloping the ventrally arcuate tail. Phasmid riblike, near middle of bursa.

Young female (fig. 2, *G*).—Length 0.7 to 0.9 mm. Width 30μ to 35μ. Vulva at 91 to 93 percent. Body practically cylindrical from esophagus to vulva. Tail convex-conoid to broad, rounded terminus, its length equal to anal body diameter. Cuticle coarsely annulated. Wings three, marked by four refractive lines, the areas occupying about one-fourth of body width. Ovary immature. Posterior uterine branch absent.

Adult female.—Length 0.8 to 1.2 mm. Width 132μ to 345μ. Spear length 20μ to 24μ. Body distorted, variable in form (fig. 2, *H*, *I*, *J*), neck and posterior end retaining a slight semblance of original form. Median bulb almost spherical with strong radial muscles. Terminal bulb merging with intestines. Body densely granular. Details of the coiled single ovary completely obscured. Vulva broad transverse slit. Anus inconspicuous. Found only in root tissues.

Diagnosis.—Anguillulina. Spear massive, strongly knobbed. Labial framework strong, cuticularized. Adult female swollen, distorted, variable in size and form, found only in roots of host. Young female of normal anguilluloid form. Vulva at 91 to 93 percent. Ovary single. Posterior uterine branch absent. Tail rounded, about as long as anal body diameter. Phasmids near terminus. Male tail about as long as anal body diameter, ventrally bent, enveloped by bursa. Phasmid riblike, inserted in bursa.

Host: Atriplex confertifolia.

LIFE HISTORY

Specimens collected December 12, 1933, were preadults with an occasional larva. The preadults pass through summer drought and winter cold, becoming active in April, when the soil is moist and warm and when shadscale growth begins. Differentiation of sexes occurs at the last molt, the males and young females being present both within the roots and in the surrounding soil. Because of the difficulty males would experience in locating females within the roots, it is probable that copulation occurs in the soil, after which the young females enter the roots. The female lengthens but little as it becomes a distorted, misshapen mass, forcing its way into the hard root tissues; the body pressure becomes so great that the nemas usually burst when the root is opened. Eggs are deposited in masses and covered with a gelatinous secretion. Segmentation and hatching occur immediately and the larvae develop into preadults, which become quiescent until revived in spring.

EFFECT ON HOST

Roots of infested bushes appear very much as if they were infested with *Heterodera marioni*, irregular swellings and occasionally typical galls being formed. As a result of infestation, many bushes are killed or severely injured. This injury is especially noticeable in years that follow a dry season. Such a condition was very evident

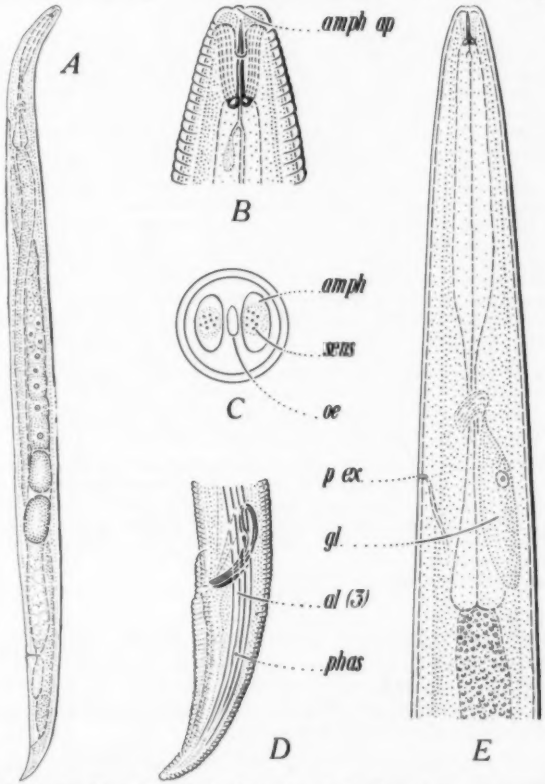

FIGURE 3.—*Neotylenchus latus: A*, Adult female, × 120. *B*, Head: *amph ap*, amphid aperture, × 1,500. *C*, Cross section of neck just posterior to spear: *amph*, amphid; *sens*, sensillae; *oe*, esophagus, × 1,500. *D*, Male tail: *al*, wings (three); *phas*, phasmid, × 500. *E*, Anterior end of female: *p ex*, excretory pore; *gl*, gland, × 500.

in 1933 after the dry year of 1931. In 1931 the precipitation was only 11.4 inches and the mean for the 5-year period 1928–32 was 13.71 inches, as compared with a general mean of 16.18 inches.

NEOTYLENCHUS LATUS N. SP.

Eight females of *Neotylenchus latus* (fig. 3) were secured from the cortex of shadscale roots near Richfield, Utah. Two males and four females were found under similar conditions in a root taken from a spot west of Utah Lake. The species appears to be a rare inhabitant of shadscale roots, too few in number to be of any significance as a parasite.

MORPHOLOGY

Measurements:

1.	11.	16.	W	[55] 85.[6]	94.5	0.7–1.1 mm
1.	3.8	4.	5.9	5.1	2.	

1.	15.	21.	[65] M	95.	0.8 mm
1.	2.2	2.4	3.2	2.	

Female.—Body attaining its greatest width at about 65 percent. Anteriorly tapers uniformly to lip region, which is not set off in any manner (fig. 3, *E*). Tail conoid to pointed terminus, its length about two and one-half times anal body diameter (fig. 3, *A*). Cuticle finely annulated. Wings three, marked by four refractive lines. Face view of lip region shows the 8 lip sections and 4 papillae characteristic of the genus.[7] Amphid apertures at apex of lateral lips (fig. 3, *B*). Amphidial pouch half as wide as head, located nearly opposite base of spear, practically invisible from lateral view. Sensilla elements apparently eight. Spear 10μ to 12μ long, with three small basal knobs. Esophagus: Corpus comprising half the total length; isthmus exceedingly slender; bulb variable, generally elongate-conoid, half as wide as neck. Large gland close to bulb (fig. 3, *E*). Excretory pore just behind nerve ring. Intestine densely granulated, generally crowded to one side by ovary. Female prodelphic, ovary outstretched, reaching almost to base of esophagus. Posterior uterine branch reaching from one-third to three-fourths the distance to anus. Eggs about as long as greatest body diameter and half as wide as long. Vulva broad, transverse slit. Anus indistinct. Phasmids slightly anterior to middle of tail.

Male.—Body much less robust than that of female. Spear normal. Testis single, outstretched. Spicula arcuate, tapering. Gubernaculum thin, flat, slightly arcuate. Bursa crenate, striated, rising about opposite middle of spicula and extending to near terminus. Tail conoid, ventrally bent, ending in small rounded terminus (fig. 3, *D*).

Diagnosis.—*Neotylenchus* with above measurements. Differs from *N. abulbosus* Steiner, 1931, in the following characters: Tail shorter; vulva located farther in front of anus; posterior uterine branch present; male with spear, spicula, and gubernaculum, normal, not undeveloped as in *N. abulbosus*.[8] *N. obesus* Thorne, 1934, is a much more obese species, with vulva located at 95 percent to almost terminal.

Host: Atriplex confertifolia.

SUMMARY

The sugar beet nematode, *Heterodera schachtii* Schmidt, 1871, is reported as an indigenous parasite of *Atriplex confertifolia* (Torr. and Frem.) S. Wats. in Utah. *Anguillulina aberrans* n. sp., and *Neotylenchus latus* n. sp., are also discussed as parasites of *A. confertifolia*, and morphological diagnoses are given.

[7] STEINER, G. NEOTYLENCHUS ABULBOSUS N. G., N. SP. (TYLENCHIDAE, NEMATODA) THE CAUSAL AGENT OF A NEW NEMATOSIS OF VARIOUS CROP PLANTS. Jour. Wash. Acad. Sci. 21: 536–538, illus. 1931.

[8] STEINER, G., and BUHRER, E. M. THE MALE OF THE NEMATODE SPECIES, NEOTYLENCHUS ABULBOSUS STEINER, AND ITS SEXUAL DIMORPHISM. Jour. Wash. Acad. Sci. 22: 482–484, illus. 1932.

SCAB OF GOLDENROD CAUSED BY ELSINOË [1]

By ANNA E. JENKINS, *associate pathologist, Division of Mycology and Disease Survey, Bureau of Plant Industry, United States Department of Agriculture,* and H. G. UKKELBERG, *in charge of Edison Botanic Garden, Edison Botanic Research Corporation* [2]

INTRODUCTION

The Edison Botanic Garden at Fort Myers, Fla., comprising about 9 acres, was established in 1927 by the late Thomas A. Edison for the purpose of finding a domestic source of rubber. Several thousand plants representing numerous genera and species were gathered from Virginia, North Carolina, South Carolina, Georgia, Florida, Alabama, Texas, and New Mexico and planted there. Collections also were made in New Jersey, and plants were contributed from many sections of the country by those interested in the project. Contributions of plants indigenous both to this country and to others were made by the United States Department of Agriculture. Goldenrod was found to have a high rubber content and was selected by Edison for further investigations. Most of the other plants were discarded, and by 1934 experimental work was being conducted with goldenrod only. The plants are arranged in beds, 6 by 20 feet, distributed over about 4 acres of the garden, with about 2 feet between the outside rows of any two beds. Each bed contains approximately 168 plants of a single species. The species represented are *Solidago altissima* L., *S. chapmanii* Gray, *S. edisoniana* Small, *S. elliottii* Torrey and Gray, *S. fistulosa* Mill., *S. leavenworthii* Torrey and Gray, *S. mirabilis* Small, *S. nashii* Small, *S. rugosa* Mill., and *S. sempervirens* L.

This paper reports a recent investigation of a hitherto unrecorded disease of goldenrod found in the garden and elsewhere in Florida.

THE DISEASE

NAME

The goldenrod disease, which is caused by an *Elsinoë*, is called "scab" rather than "anthracnose" because of its hyperplastic nature. This is in accordance with a previous suggestion (*8*) [3] that diseases of this character be referred to as "scab."

HISTORY, RANGE, AND SPECIES AFFECTED

Scab of goldenrod was discovered first in the Edison Botanic Garden in June 1933. The disease was noted on young plants and on the young leaves and stems of older plants of *Solidago edisoniana*. Further examination revealed that *S. sempervirens* and *S. fistulosa* also were affected. Lesions on the lower part of the plants indicated that the disease had been present early in the season.

[1] Received for publication July 18, 1935; issued December 1935.

[2] The writers are indebted to L. G. Polhamus, of the Division of Plant Exploration and Introduction, Bureau of Plant Industry, U. S. Department of Agriculture, for cooperation in various ways, including the examination of goldenrods in certain localities for evidence of the disease. Thanks are also due W. M. Buswell, of the University of Miami, Coral Gables, Fla., for the contribution of specimens of *Solidago* from his private herbarium, on the basis of which certain historical facts relative to the disease were established.

[3] Reference is made by number (italic) to Literature Cited, p. 525.

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In 1934 lesions were first observed late in April on young plants of *Solidago sempervirens* and somewhat later on *S. fistulosa*. During July the disease appeared in a few beds of *S. edisoniana*, at first affecting only a small number of plants, but gradually spreading throughout these beds and to several others of this species. Young plants transplanted in June and July were severely attacked and some were killed. In one instance, in August, a few lesions were found on plants of *S. leavenworthii* growing in pots in a slat house. These were limited to the leaf bases, regions of the goldenrod plant extremely susceptible to infection. Throughout the season the disease continued to be prevalent and was often severe on *S. edisoniana*, *S. fistulosa*, and *S. sempervirens* (pl. 1; pl. 2, *A;* pl. 3, *A–D*). *S. elliottii* and *S. mirabilis* also were moderately attacked.

The distribution of scab on goldenrod growing wild in Florida in Polk, Hardee, De Soto, Charlotte, Lee, and Dade Counties (fig. 1) was established through limited surveys made during the autumn of 1934. At Fort Myers Beach, about 18 miles from Fort Myers, and nearby at Punta Rassa, *Solidago sempervirens* was affected by the disease and in some cases severely so (pl. 4, *A* and *B*). Other species, namely, *S. chapmanii*, *S. fistulosa*, *S. stricta* Ait., and *S. tortifolia* Ell., growing in the same locality were not affected.

From Fort Myers northward to Fort Meade, a distance of about 100 miles, *Solidago sempervirens* was found in two different localities near Punta Gorda, and in each case all of the plants were affected. South of Fort Meade was found practically a pure stand of *S. fistulosa* covering approximately 8 acres, and here it was estimated that about 35 percent of the plants were affected. The disease also was present on a few plants of *S. edisoniana* in the vicinity of Wauchula and Fort Meade, the latter being the type locality for the species (*14*). *S. chapmanii*, *S. minor* (Michx.) Fernald, and *S. tortifolia*, growing in the section surveyed from Fort Myers to Fort Meade, were disease-free.

In Dade County scab was prevalent on *Solidago sempervirens* (pl. 5, *I*) growing wild in the vicinity of the United States Plant Introduction Garden at Coconut Grove when examined [4] for the disease in October 1934.

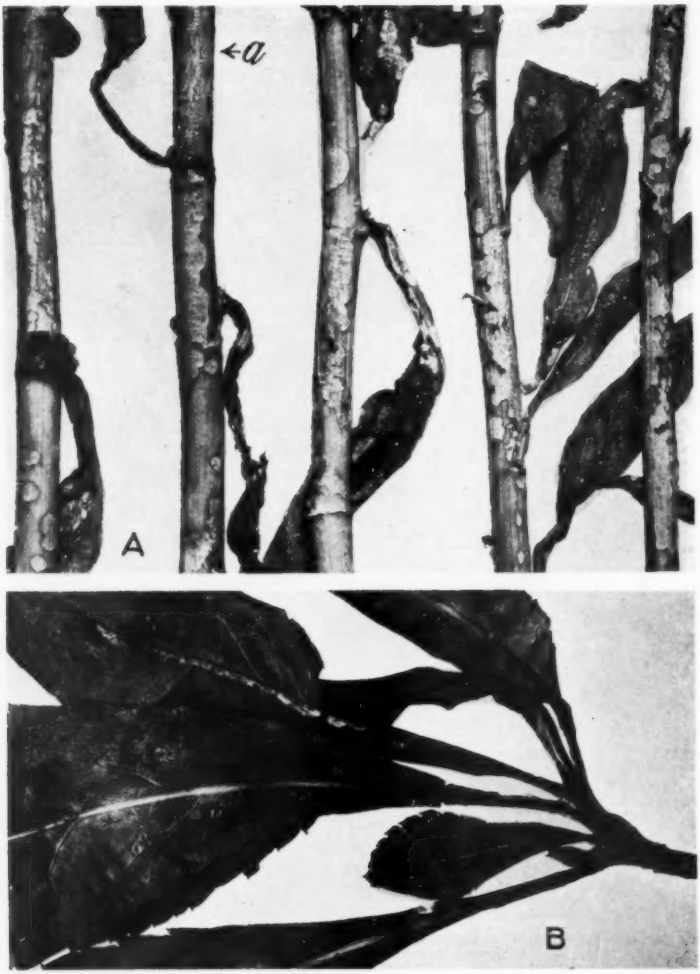
Additional records of scab in Florida, and among them the earliest available, are provided by specimens of *Solidago* from the Buswell collection, which were contributed in order that they might be examined for possible scab lesions. These are present in greater or less abundance on *S. edisoniana* (pl. 2, *B*), *S. elliottii*, *S. fistulosa*, *S. mirabilis*, and *S. sempervirens* collected in the Edison Botanic Garden on October 31, 1930, December 6, 1933, and intermediate dates, and on wild plants of *S. edisoniana* from Polk County collected on November 9, 10, and 28, and December 3, 1932. A specimen of *S. chapmanii* bearing no date or place of collection in Florida also shows severe infection.

EXPLANATORY LEGEND FOR PLATE 5

A–H, Leaves of *Solidago sempervirens* affected by scab. *A*, *a–c*, Upper surface of young leaves; *B*, *a–c*, their lower surfaces; *C*, *a* and *b*, upper, and *D*, *a* and *b*, lower surface of two older leaves; *C*, *c*, and *D*, *c*, lesion penetrating leaf base; *E*, numerous lesions on upper side of leaf; *F*, elongate lesion involving midrib; *G*, leaves dead or dying from scab; *H*, *a*, young blighted leaves on an adventitious shoot. (All × 1.) From Edison Botanic Garden, June 21, 1934. *I*, Scab lesions on wild *Solidago sempervirens* (× 1); *J*, pycnidium of an unidentified fungus on stem lesions of *S. edisoniana*, Edison Botanic Garden, October 17, 1934 (× 475).

[4] Examination made by L. G. Polhamus.

Upper part of diseased plants of *Solidago sempervirens* from Edison Botanic Garden, Fort Myers, Fla., June 21, 1934: *A*, *a*, and *B*, *a*, Regions of extremely severe infection; *A*, *b*, adventitious shoots; *B*, *b*, leaves dying at the base. × about ½.



A, Lower part of stems shown in plate 1, or of those from same source; *a*, russeted region. *B*, Scab lesions on young plant of *Solidago edisoniana* representing a phanerogamic specimen from the Edison Botanic Garden collected November 28, 1932. × 1.

The data thus indicate that scab was prevalent on wild goldenrod in Florida for a long period before the Edison Botanic Garden was established. Indirectly the discovery of the disease at the present time may be attributed to the special attention given goldenrod in the garden upon the discovery of its high rubber content, together with the fact that the garden was located within what is now known to be the geographic range of the recently discovered disease. Ecologically this general section is highly favorable to the development of other diseases similar to scab of goldenrod. An example is scab of citrus (*Citrus*), the environmental relations of whose causal fungus (*Sphaceloma fawcettii* Jenkins) have been the subject of considerable investigation (*5, 10, 12, 16*).

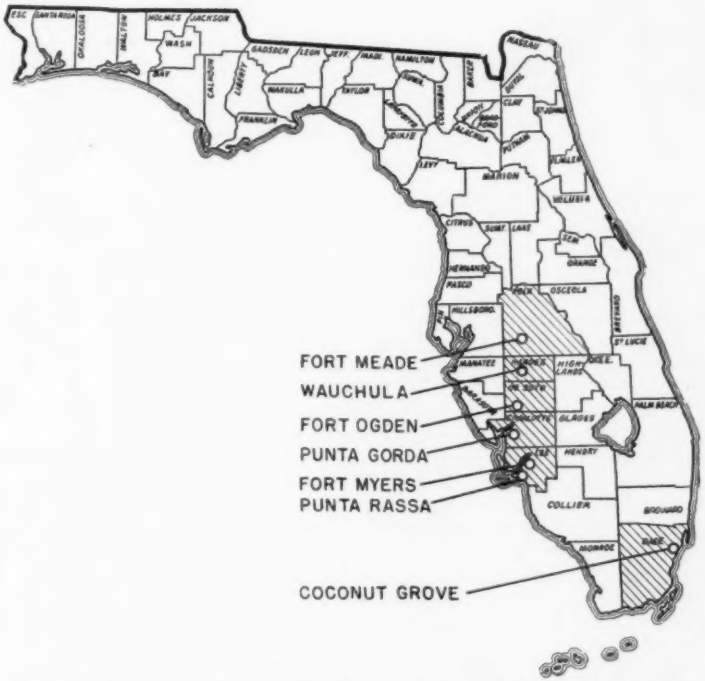


FIGURE 1.—Map of Florida, showing (by shading) the counties in which scab occurs on wild goldenrod, and also the location of towns in or near which the disease was observed.

When examined in July 1934 no scab was found on plantings of goldenrod maintained by the United States Department of Agriculture at Charleston, S. C., and at Savannah, Ga. The plantings at Charleston were again examined for scab early in October,[5] but no evidence of the disease was found. In the summer of 1935, however, the disease was found on *Solidago sempervirens* at Savannah Beach, Ga., and on this species and *S. fistulosa* growing wild near the Barbour

[5] Examination made by L. G. Polhamus.

Lathrop Plant Introduction Garden, Savannah, Ga. In the plantings at the garden itself *S. edisoniana*, *S. fistulosa*, *S. leavenworthii*, *S. sempervirens*, and *S. serotina* were all found badly attacked.

IMPORTANCE

Rubber is formed in the leaves of goldenrod (*13*) and increasingly so as they mature (*1*). The stunting or killing of young plants by scab results, of course, in a potential loss of leafage, and there is also a direct loss from the killing of the leaves themselves. With respect to *Solidago sempervirens* the loss of leafage from the ravages of the disease is difficult to estimate, because normally some of the leaves die before the plant reaches maturity. Cases have been noted, however, in which most of the leaves were definitely killed by scab The leafage loss of severely diseased plants of *S. edisoniana* and *S. fistulosa* is estimated to be from 5 to 10 percent. Plants may be severely infected and still remain alive, but whether the rubber content of the diseased leaves is affected has not been determined.

SYMPTOMATOLOGY

Morphologic Symptoms

Scab of goldenrod attacks the young growth of the plant above the ground. Young plants may be killed or stunted, as already mentioned, but ordinarily they grow to maturity more or less as usual, the new growth becoming infected as it develops (pl. 1; pl. 2; pl. 3, *A–D*). Occasionally the terminal bud is killed, preventing further growth of the axis except by the production of an adventitious shoot from below, as sometimes occurs. Some of the leaves wither and die as they unfold, while others that expand later succumb (pl. 1, *B*, *b;* pl. 5, *G*), especially if the highly susceptible basal part (pl. 5, *A* and *B*) is affected; in many cases, however, owing to the hyperplastic nature of the disease, the affected leaves are not killed.

Similarly, the stem usually remains alive even though girdled for several centimeters or when as much as four-fifths of its surface is covered with lesions. There may be merely a russeting of the stem (pl. 2, *A*, *a*). All or most of the leaves produced during periods extremely favorable for infection may be killed, so that the stem is practically devoid of green leaves for several centimeters (pl. 1, *A*, *a*, and *B*, *a*). Where the leaves are lost adventitious shoots may be forced into development only to become infected in turn (pl. 1, *A*, *b;* pl. 5, *H*).

ON SOLIDAGO SEMPERVIRENS

The leaf spots on the highly susceptible species of *Solidago sempervirens* are larger and more striking, as well as often more numerous, than on the other species. They occur anywhere on either side of the leaf (pl. 5, *A–F*), although somewhat more frequently on the lower surface. Midribs, veins, and petioles as well as the basal part of apetiolate leaves are often affected (pl. 4; pl. 5, *A–D*; pl. 6, *B*, *a*). Lesions on the bases of apetiolate leaves (pl. 5, *C*, *c*, and *D*, *c*) and those occurring elsewhere on large succulent leaves (pl. 6, *B*) may discolor both leaf surfaces, but generally they are visible on one leaf surface only (pl. 5, *A*, *a*, *b*, *c*, and *B*, *a*, *b*, *c*; *C*, *a*, and *D*, *a*; *C*, *b*, and *D*, *b*). Lesions may extend for a greater or less distance from the base

Scab lesions on (*A*) leaves of *Solidago fistulosa*, (*B*) on stem of *S. ediscniana*, and on (*C*) stem and (*D*) inflorescence leaves of *S. sempervirens*. Material from Edison Botanic Garden, *A–C* collected August 4 and *D* October 17, 1934. *E–G*, Agar slant cultures of the *Elsinoë* causing the disease, approximately a month old, on (*E*) potato dextrose, (*F*) corn meal, and (*G*) beef. All × 9/10. Drawings by J. Marion Shull.

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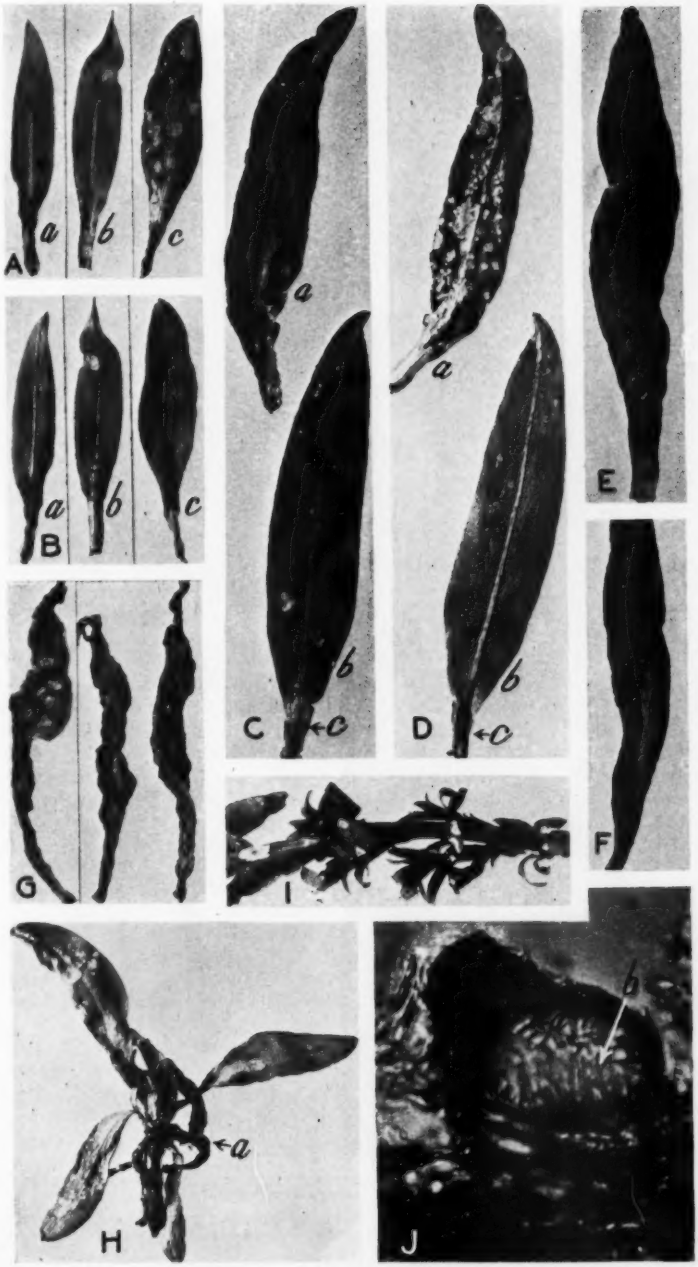
Wild *Solidago sempervirens* affected by scab: *A* From Punta Rasa, August 17, 1934; *B* from Fort Myers Beach, August 14, 1934. *A*, *a*, Base of stem; *B*, *a*, upper part of same plant. Photographs from dry specimens. × 1.



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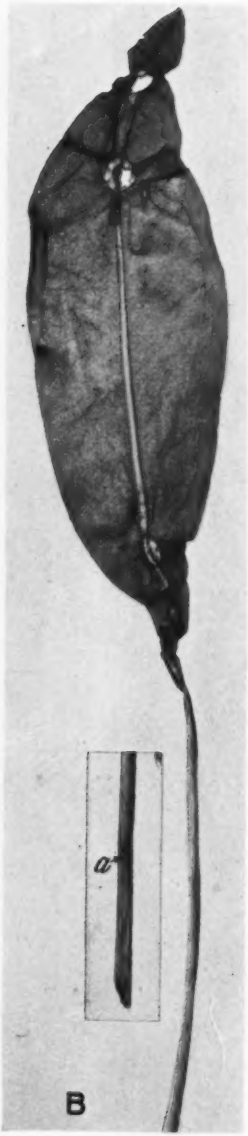

A, *a*, Ascomata on stem lesions of *Solidago sempervirens* (× 7); *B*, blade and (*a*) petiole lesions on the same species resulting from artificial inoculations with the *Elsinoë* (× 1).

toward the apex of the leaf (pl. 4); those on the lower or dorsal leaf surface may be continuous with stem lesions. The more restricted leaf lesions range from punctiform areas to circular, subcircular, or irregular spots reaching 5 mm in diameter (pl. 4; pl. 5, *A–F*). Lesions may be slightly raised at first, and later depressed on one leaf surface and bulged on the other (pl. 5, *A*, *b*, and *B*, *b*). The entire leaf may be bent away from the point of infection. In the beginning the lesions appear as brown, often "madder brown",[6] water-soaked spots. The center and soon the entire lesion, or all except a narrow bordering line or edge, becomes "vinaceous buff." This coloration finally becomes white or gray.

On stems of *Solidago sempervirens* the lesions are of essentially the same coloration as on the leaves. Sometimes they are surrounded by a narrow dark line, and they may be slightly raised, especially when old (pl. 3, *C*). The smaller spots are often circular, subcircular, or irregular, and 8 ± mm in diameter; occasionally they are more or less linear. Larger lesions, sometimes formed by the fusion of smaller spots, are often extremely irregular in shape. They may be unevenly scalloped or variously lobed, and they sometimes enclose green patches of the stem. Transverse cracks may appear on the surface of fairly young lesions still "vinaceous buff" in color (pl. 2, *A*).

ON SOLIDAGO EDISONIANA AND OTHER SPECIES

Although similar to those of *Solidago sempervirens*, the scab lesions on *S. edisoniana* differ in certain respects. The leaf spots are often circular to subcircular, 2 to 3 mm in diameter (pl. 2, *B*). They are generally reddish at first, then more or less permanently "hazel" or "brick red", although in some cases they are paler or even white. Sometimes they discolor both leaf surfaces. The leaf spot may become perforated or fall entirely away, or only the cuticle on one leaf surface may remain. The stem lesions are similar to the leaf lesions (pl. 3, *B*), but when old they may be somewhat paler and bordered by purple.

The lesions of *Solidago elliottii* and *S. mirabilis* are similar to those of the closely related species *S. edisoniana*, while those of *S. fistulosa* (pl. 3, *A*) are somewhat intermediate between those of *S. edisoniana* and *S. sempervirens*.

Signs

The signs of goldenrod scab are primarily the fruit bodies of the perfect stage of the pathogen, which may be relatively inconspicuous. These ascomata are present from as early as June until the end of the season, and they appear as small dark more or less convex areas (pl. 6, *A*, *a*) representing the epithecial covering (pl. 7, *B*, *a*, and *F*, *b*). For the most part they are confined to mature or old stem and leaf-base lesions. Small black specks occasionally present on old lesions (pl. 5, *J*) are pycnidia or pycnidialike structures, but it has not yet been possible to determine definitely whether they represent a conidial stage of the causal fungus. In some cases mature lesions are darkened by a thin layer of superficial dark hyphae or other vegetative development of the fungus (pl. 7, *A*).

[6] The color readings given in quotations are by J. Marion Shull and are based on the following publication: RIDGWAY, R. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 pp., illus. Washington, D. C. 1912.

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Histologic Symptoms

As in related diseases, the lesions are characterized by the formation of what has been referred to by Butler (*3, pp. 180–181*) as "wound cork." This development in leaf lesions of citrus scab has been described in detail by Cunningham (*4*). Based on Cunningham's account and on prepared mounts which he gave Butler, the latter (*3, p. 181*, and *pl. 8, figs. 6 and 7*) has summed up the situation as follows:

The pathogen—

causes a necrosis of a few of the superficial cells of the leaf and provokes the active division of the spongy parenchyma. The new walls are roughly parallel, in the early stages, to the necrosed surface and the process extends to a considerable depth, sometimes leading, in attack on the under surface, to divisions in the palisade cells on the opposite side. Near the lesion the cells elongate more towards the injured part and become more divided than those farther away, and in the layer of long cells with several cross walls a cork cambium eventually develops * * *. This extends up to the epidermis all around the lesions and cuts off cork on its outer side and a little phelloderm on the side toward the sound tissues. All the primary cell walls of the hyperplastic area are thickened and intercellular spaces are much reduced.

THE CAUSAL FUNGUS

CLASSIFICATION

The ascomycete causing scab of goldenrod is a previously unreported member of the genus *Elsinoë* Rac., and its imperfect stage is typical of the form genus *Sphaceloma* De Bary. As previously shown (*6*), this genus belongs to the Myriangiales, which are typified by *Myriangium duriaei* Mont. and Berk. *Elsinoë* is typified by *E. canavaliae*, which causes scab of swordbean (*Canavalia gladiata* (Jacq.) DC.). A closely related and actually better known species, however, is *E. phaseoli* Jenkins (*2*), in connection with the study of which certain new features pertaining to the morphology of the genus (*7, 9*) were demonstrated, such as the double-walled character of the ascus (*7*). Available literature relative to the history and relationships of the order were reviewed by the writer in connection with the discussion of *E. canavaliae* (*6*). Tai (*15*) has since discussed the relationships of the order on the basis of his study of the development of *Myriangium bambusae* Rick. He interprets the outer sheath or primary wall of the ascus as homologous with the perithecial wall in the Sphaeriales and also with the rudimentary wall (*11*) of the ascocarp in *Catacauma flabellum* (Schw.) Theiss. and Syd. of the Dothideales.

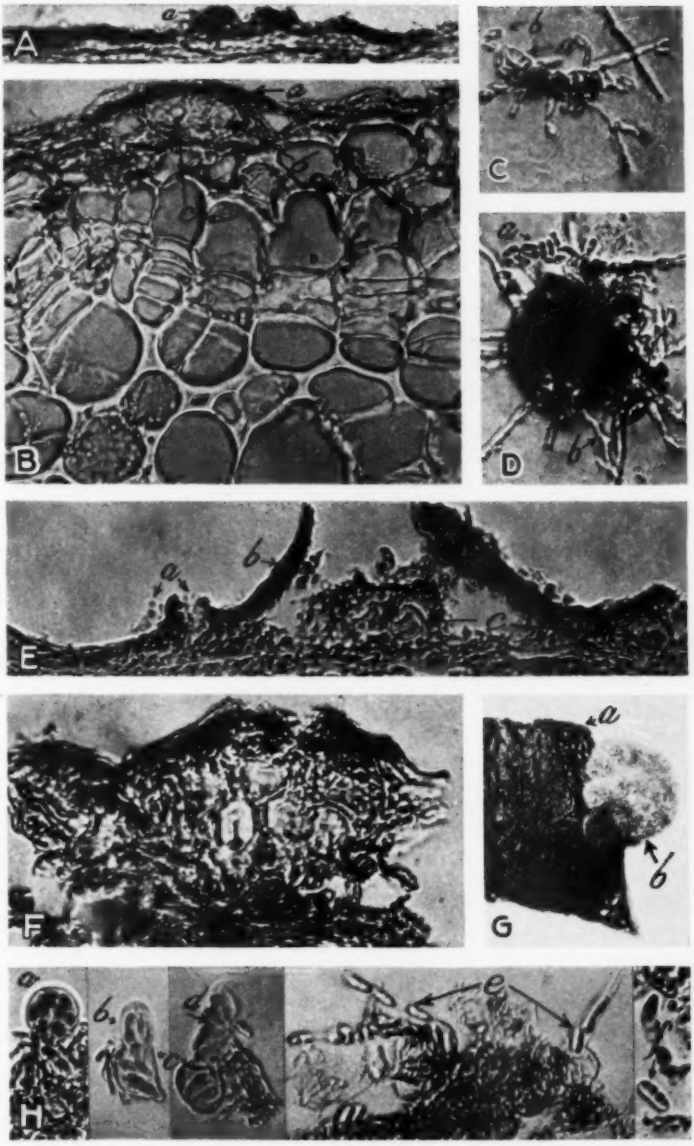
MORPHOLOGY

Perfect Stage

Rather recently formed ascomata (pl. 7, *B*) of the goldenrod *Elsinoë*, some containing as many as 15 asci, were present on a few of the oldest lesions of the upper 20 to 25 cm of stems collected in the

EXPLANATORY LEGEND FOR PLATE 7

Elsinoe solidaginis: A, Surface of old stem lesions darkened by superficial growth of the fungus; *a*, possibly an endospore. *B*, Section of a lesion shown in plate 6, *A*, showing a young ascoma; *a*, epithecium; *b* and *c*, asci; *d*, hyperplastic area of lesion (× 375). *C* and *D*, Production of conidia in young culture from an old hyphal fragment and from a small mycelial mass; *C*, *a*, conidiophore; *C*, *b*, and *D*, *a*, conidia; *D*, *b*, young hyphae (× 400). *E–F*, Sections of outer part of stem lesions showing more mature ascomata than the ascoma represented in *B*; *E*, *a*, cavity or pocket containing hyaline spherical conidia; *E*, *b*, epithecium; *E*, *c*, asci. (*E* × 350; *F* × 475). *G*, Tissue planting (*a*) and culture growing from it (× 10). *H*, maceration of ascomata from stem lesion showing; *a*, ascus, with both walls intact, although line of outer wall is not visible in the photograph; asci whose inner elastic walls (*b*) have expanded following rupture of the (*c*) outer inelastic walls; *d*, ascospores within ascus; *e*, still greater expansion of inner part of ascus, from whose apices several spores have been discharged; (*f*), 1- and 3-septate ascospores (*a* × 440; *c* × 450; *e* × 330; *f* × 440.)



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Edison Botanic Garden on June 1, 1934 (pl. 6, *A*, *a*). Older ascomata from specimens collected in August are shown in plate 7, *E* and *F*, and asci and ascospores in plate 7, *B*, *b* and *c*; *E*, *c*; and *H*. *H*, *a*, represents an ascus of which the outer sheath is probably about to rupture; *H*, *b*, partial expansion of the ascus after the rupture of this sheath; and *H*, *d–f*, ascospores still within the expanded asci or discharged from them.

A feature of the ascospore of *Elsinoë*, first observed in the case of *E. phaseoli* [7] and also noted on the goldenrod *Elsinoë*, is the rupture of the wall of the ascospore allowing the protoplasmic contents of one or more cells to escape in sporelike masses.

Conidial Stage

Tufts of hyaline conidiophores representing the *Sphaceloma* stage of the goldenrod fungus were found in certain sections of young "madder brown" lesions on *Solidago sempervirens*. These evidently were produced from more or less horizontal subcuticular or intraepidermal hyphae after the manner illustrated in plate 7, *C*, showing conidial formation within a 24-hour period, from a fragment of an old hypha. The culture was made by smearing small masses of an old culture on the surface of a thin film of corn-meal agar on a glass slide. To provide a moist atmosphere, the new culture was placed in a Petri dish lined with filter paper. Plate 7, *D*, shows additional new conidia (*a*) and also a young hypha (*b*) produced from a small mycelial mass transferred to the fresh agar substrate. There was soon conidial formation from the new hyphae, the conidia in some instances being sessile on the hyphae. The conidia just described were generally ovoid or elongate-elliptical, and often biguttulate, measuring 6.5 to 8.6μ by 2.6 to 4μ. Some of the conidia germinated practically as soon as formed, either by the production of germ tubes or sprout conidia. Spherical conidia at first minute but enlarging to at least 3μ in diameter occur singly or in agglutinated masses. Apparently they are produced from almost any structure of the fungus, and as here illustrated (pl. 7, *E*, *a*) they appear to have formed within the epithecium. The spherical body shown in plate 7, *A*, *a*, is possibly an endospore.

The dark, membranous pycnidium apparently of the *Elsinoë* shown in plate 5, *J*, was produced subepidermally; the conidia (*a*) and conidiophores (*b*) within it resemble those shown in plate 7, *C* and *D*. Other more or less similar membranous structures, often lenticular in shape, were seen in or on the lesions.

Identity

It appears that an *Elsinoë* has not been reported on goldenrod or other Compositae heretofore. The asci and ascospores are somewhat smaller than those of other species that have been described, and the organism is culturally distinct from the members of this genus and of *Sphaceloma* so far as isolated. The name *Elsinoë solidaginis* is therefore proposed for the fungus, and the following technical description is given.

[7] Unpublished data.

Technical Description

Elsinoë solidaginis, n. sp.

Hyphae subcuticular, intraepidermal, or subepidermal, i. e., in the tissue outside of the wound cork, hyaline, or dark, sometimes chainlike, up to at least 6μ in diameter, sometimes forming more or less effuse stromata reaching 15μ in thickness; ascomata apparently intraepidermal for the most part, punctiform, reaching 150μ in surface diameter and 50μ in thickness; dark-colored epithecium up to 20μ thick, becoming ruptured and exposing underlying ascogenous layer; unruptured asci mostly spherical to obpyriform, 15 to 17μ × 15 to 18μ, occasionally with a slight stipe; ascospores colorless, 2- to 3-celled, 8 to 13μ × 4 to 5μ, protoplasmic cellular contents sometimes escaping as sporelike granular masses; conidial stage of the form genus *Sphaceloma*, conidiophores as observed in culture often more or less conical, 1- to 2-septate, bearing conidia acrogenously, sometimes several in succession; conidia ovoid to oblong-elliptical, hyaline, often biguttulate, 1-celled, 6.5 to 8.6μ × 2.5 to 4μ, germinating by means of a germ tube or by the production of sprout conidia; spherical conidia produced from various structures of the fungus, of various sizes and with thick, viscid covering, often forming agglutinated masses.

Ascomata punctiformia, usque 150μ diam. ad superficiem et 50μ in crassitudine; asci infracti sphaerici vel obpyriformes, 15–17μ × 15–18μ; ascosporae 2–3 cellulares, 8–13μ × 4–5μ; status conidiophorus ad formam-generem *Sphacelomatem* pertinens; conidiophora (in culturis) saepe plus minusve conica 1–2-septata; conidia acrogena, ovoidea vel oblongo-elliptica, unicellularia, 6.5–8.6μ × 2.5–4μ; conidia sphaerica usque 3μ diam., e structuris variis fungi nata, massas agglutinatas formantia.

Hosts.—On wild and domesticated species of *Solidago chapmanii*, *S. edisoniana*, *S. elliottii*, *S. fistulosa*, *S. leavenworthii* (rarely), *S. mirabilis*, and *S. sempervirens*, producing the disease known as scab, hyperplastic in nature, infecting tender young growth, sometimes killing it, but often producing more or less extensive diseased areas on the surface of stems and leaves; lesions of various sizes and shapes, on *S. sempervirens* generally "vinaceous buff", becoming white or nearly so; on *S. edisoniana*, *S. elliottii*, and *S. mirabilis*, often "hazel" to "brick red", occasionally white or whitish; on *S. fistulosa* of intermediate coloration.

Distribution.—Florida: Polk, Hardee, De Soto, Charlotte, Lee, and Dade Counties.

Specimens examined.[8]—Florida: On *Solidago chapmanii*, W. M. Buswell (date not given).[9]

On *S. edisoniana*, Hardee Co., Wauchula, H. G. Ukkelberg, Sept. 30, 1934; Lee Co., Fort Myers, Edison Botanic Garden, W. M. Buswell, Nov. 28, 1932 *, Aug. 17 and Nov. 10 *, 1933; H. G. Ukkelberg, July 9, Aug. 4 (type), and Oct. 17, 1934; Feb. 6, 1935; Polk Co., W. M. Buswell, Nov. 9 *, 10 *, and 28 *, and Dec. 3 *, 1932; Fort Meade, H. G. Ukkelberg, Sept. 30, 1934.

On *S. elliottii*, Lee Co., Fort Myers, Edison Botanic Garden, W. M. Buswell, Dec. 4 * and 6 *, 1932, and Dec. 6, 1933 *.

On *S. fistulosa*, De Soto Co., Fort Ogden, H. G. Ukkelberg, Sept. 20, 1934; Lee Co., Fort Myers, Edison Botanic Garden, W. M. Buswell, Nov. 10, 1930 *, and May 10, 1933 *; H. G. Ukkelberg, Aug. 4 and Oct. 17, 1934; Feb. 6, 1935.

On *S. mirabilis*, Lee Co., Fort Myers, Edison Botanic Garden, W. M. Buswell, Dec. 4, 1933 *.

On *S. sempervirens*, Charlotte Co., Punta Gorda, H. G. Ukkelberg, Sept. 30, 1934; Dade Co., Coconut Grove, L. G. Polhamus, Oct. 1934 (specimen represented by photographs); Lee Co., Fort Myers, Edison Botanic Garden, W. M. Buswell, Oct. 31, 1930 *; H. G. Ukkelberg, June 1, 21, July 9, and 28, Aug. 4, Oct. 17, and Oct. 26 (infection from artificial inoculation in greenhouse), 1934; Feb. 6, 1935; vicinity of Fort Myers, W. M. Buswell, Oct. 24, 1931 *; H. G. Ukkelberg, Aug. 14, 1934 (beach); Punta Rassa, H. G. Ukkelberg, Aug. 17, 1934.

District of Columbia: On *S. sempervirens*, A. E. Jenkins, Oct. 12, 1934 (infection from artificial inoculation) in laboratory.

[8] The specimens examined are deposited in the mycological collections of the Bureau of Plant Industry, U. S. Department of Agriculture, and a living culture of the fungus (culture no. 430 A. E. Jenkins) has been sent to the Centraalbureau voor Schimmelcultures, Baarn, Netherlands.

[9] The specimens marked by an asterisk represent specimens from the Buswell collection on which lesions of scab were found during the course of the present study.

CULTURAL CHARACTERISTICS

Pure cultures of the fungus were obtained from isolations made from brown lesions on young leaves of *Solidago sempervirens* collected in the Edison Botanic Garden on July 6. For the first isolations, made on July 11, scrapings from the surface of the lesions were plated on corn-meal agar. By July 25 several thalli characteristic of the genus *Elsinoë* were visible in the six plates that were made. The circular, glistening growths were just becoming colored. The largest thallus, measuring about 2 mm in diameter, was transferred to a potato-dextrose agar slant. When observed about a week later it had increased in size to about 3 mm. The margin was then raised and was "pinkish cinnamon" in color; the central region was depressed and was "cinnamon" in color.

On July 12 additional isolations were made from the specimens collected on July 6 by dipping pieces of young leaves and stems in mercuric chloride (1:1000), rinsing them in sterile water, and then planting them on the surface of corn-meal agar plates. Plate 7, *G*, shows one of the thalli obtained from these plantings.

More abundant cultural growth was obtained by cutting goldenrod stems into short lengths, treating them as for the leaf plantings, and then transferring them to test tubes containing a small amount of sterile water. When examined after 3 weeks some of the lesions were covered with a mat of fungus, consisting chiefly of conidia, including swollen and germinated conidia of various sizes. Transfers of this growth failed to yield pure cultures, but platings would doubtless have given many pure thalli of the fungus.

In a set of parallel cultures of the *Elsinoë* grown for approximately a month on agar slants of potato dextrose, corn meal, and beef, the coloration of the fungus was generally "maroon" to nearly black, "bay", and "clay color", respectively. Similar cultures on glycerin agar slants were "pale drab-gray" at the center and "maroon" to black in the peripheral region.

INOCULATION EXPERIMENTS

At the Edison Botanic Garden artificial inoculations were made on September 20 on young potted plants in a greenhouse in which the temperature was not controlled. The species inoculated were *Solidago altissima* and *S. leavenworthii*, which are highly resistant or immune to scab, and the two highly susceptible strains *S. fistulosa* and *S. sempervirens*. *S. sempervirens* is more susceptible than *S. fistulosa*, as already explained, and the two strains of these species employed were selected for their extreme susceptibility. The inoculum consisted of the culture isolated from *S. sempervirens* on July 11, grown for several weeks on potato-dextrose agar.

Several inoculations were made on each species or strain by the cotton-plaster method employed by Winston (*16, p. 23*) in similar inoculations, i. e., the cultural growth was macerated and placed on young leaves with the fungus in contact with the leaf. The whole was then covered with paraffin-paper hoods gathered and tied in such a way as to prevent the drying out of the cotton pads. The inoculated growth was then tied to a stake to hold it in position and to prevent the weight of the wet cotton from breaking the plant. The

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paraffin paper and the cotton bearing the inoculum were removed after 48 hours.

In another experiment a few plants of each of the species and strains mentioned were atomized with a water suspension of a culture and held for 48 hours in a moist chamber before being removed to the greenhouse bench. In this experiment, as in the other, the check plants received the same treatment as those sprayed except that no inoculum was used.

The conditions of the experiments were apparently rather unfavorable for infection for, so far as could be detected, scab lesions were produced only on *Solidago sempervirens* inoculated by the cotton-plaster method (pl. 6, *B*). The lesions were small and "madder brown" when first observed on October 9. By October 20 they were "vinaceous buff" and the largest were 5 mm in diameter.

At Washington, D. C., under weather conditions believed to be favorable for infection, young inflorescence leaves inoculated by the cotton-plaster method became infected chiefly on the leaf bases. The checks remained healthy.

CONTROL

In the garden one bed of *Solidago sempervirens* was sprayed with bordeaux mixture (4–4–50) during May and June. Three applications were made at 2-week intervals, with no apparent checking of the disease. During July, August, and September, lime-sulphur was applied weekly on a series of six beds of *S. edisoniana*. In this case the amount of the disease was not reduced at first, but in the latter part of the experiment partial control was obtained. In order to obtain control by spraying, frequent applications of a protectant from practically the beginning of the growing season probably would be necessary.

It appears that selection for resistance may prove an effective means of control. Variation in the degree of susceptibility of different clonal lines obtained from rootstock cuttings was observed in 1934, particularly in the case of *Solidago edisoniana*. For example, plants of the same clones were heavily infected while other clones in nearby rows were free from lesions, or nearly so, although they were in contact with heavily infected growth. In some instances strains of *S. fistulosa* and *S. sempervirens* introduced into the garden from different localities and differing somewhat in vegetative characters have exhibited different degrees of susceptibility. The Edison selection *S. leavenworthii* is highly resistant, as previously indicated.

SUMMARY

Scab of goldenrod, hitherto unreported, was discovered in the Edison Botanic Garden, Fort Myers, Fla., in June 1933. Limited surveys made in Florida during the autumn of 1934, together with an examination of herbarium specimens, revealed the fact that the disease was present in the garden as early as 1930, and that it occurs on wild goldenrod in Polk, Hardee, DeSoto, Charlotte, Lee, and Dade Counties. The species known to be susceptible are *Solidago chapmanii*, *S. edisoniana*, *S. elliottii*, *S. fistulosa*, *S. leavenworthii*, *S. mirabilis*, and *S. sempervirens*. Of these, *S. edisoniana*, *S. fistulosa*, and *S. sempervirens* are highly susceptible and *S. leavenworthii* slightly so.

The disease results, directly or indirectly, in the loss of the leafage in which rubber is formed.

The symptoms of the disease are described.

The causal fungus belongs to the genus *Elsinoë*. The morphology and cultural characteristics of the organism are given, and it is described as a new species, *E. solidaginis*. Artificial inoculations on *Solidago sempervirens* gave positive results.

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OXIDATION AND GAS FORMATION IN THE SPONTANEOUS HEATING OF HAY [1]

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INTRODUCTION

In a series of large-scale experiments on the spontaneous heating of hay, conducted by the Bureau of Chemistry and Soils, hay was stored under varying conditions in order to determine those conducive to excessive heating and ultimately to spontaneous ignition, as well as to investigate the causes and effects of spontaneous heating under these conditions.

In these experiments the progress of heating was followed carefully, observations of various factors that could influence spontaneous heating were made, and the products of such heating were investigated.

The results of analyses of the gases formed during the heating of hay in these experiments showed a striking similarity to the results of a laboratory study designed to determine the relative tendency of undecomposed and decomposed hay to absorb oxygen. For this reason both investigations are presented in this paper.

Conflicting hypotheses have been proposed by investigators to account for the spontaneous heating and ignition of hay. However, it is commonly accepted that the production of heat in a mass of undercured or wet hay at temperatures up to 70° C., or slightly higher, is due mainly to the respiration process of the living plant cell and the activity of micro-organisms. But to account for temperatures above this, at which micro-organisms are no longer active, causes other than biological are ascribed. These are generally referred to as chemical.

The extent to which heating during the period of biological activity may be ascribed to purely chemical action has been the subject of considerable investigation and debate. It has been held by proponents of chemical-activity theory that if the higher temperatures are to be ascribed to chemical or physical agencies, it is quite reasonable to suppose that these agencies already have begun to operate at the lower temperatures. Since the results presented in this paper are concerned primarily with temperatures lying within or slightly above the range ascribed to biological agencies, a brief review of investigations supporting the theory of chemical action at these temperatures is given as a basis for the discussion of the results.

[1] Received for publication June 5, 1935; issued December 1935.

[2] This paper includes the results of 1 phase of the general investigation of the spontaneous heating and ignition of agricultural products conducted by the Chemical Engineering and Food Research Divisions, Chemical and Technological Research. The author acknowledges with thanks his indebtedness to C. A. Browne, principal chemist in charge of research; to D. J. Price, principal engineer in charge, Chemical Engineering Division; and to his associates in the conduct of the large-scale spontaneous-heating experiments, for the use of data pertaining to these experiments. To H. E. Roethe, engineer; M. A. Bradshaw, assistant engineer; and E. D. Gordon, formerly assistant engineer, Chemical Engineering Division, the author is indebted for assistance in the collection of the gases investigated.

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REVIEW OF PREVIOUS INVESTIGATIONS

Miehe (*6*, *7*)[3] held that beyond the limited effect of plant respiration the rise of temperature is due mainly to the action of micro-organisms. This action could carry the temperature up to 70° C., or slightly higher, but he found that at 68.5° all micro-organisms had been destroyed. He opposed the view that ordinary heating in haymows is due to simple chemical activity, but suggested that when the temperature rose above approximately 70° some physical or chemical change had been produced by the long-continued effect of heat or bacterial action at 70°, which rendered the hay susceptible to purely chemical oxidation. He suggested further that hay carbonization might occur by a process of dry distillation even at a temperature as low as 70° to 80°, if this temperature were maintained for a considerable time.

According to Burri (*3*), in the processes of spontaneous heating the temperature rarely exceeds 70° C., beyond which the activity of enzymes and micro-organisms is absolutely excluded. If the temperature can be raised beyond 70° by purely chemical processes it would appear plausible that the same agencies are already active at lower temperatures, even between 50° and 70°.

He held that there were good reasons for believing that, in the case of hay which is sufficiently wet, and in which the living plant has died and the respiration process has ceased with the production of a temperature of 45° to 50° C., a further increase in temperature can be produced by a chemical oxidation process, but that this chemical process alone would probably not be of sufficient intensity and duration to raise the temperature to 70°. However, on the assumption that in haymaking the respiration enzymes have been rendered more resistant to heat than is commonly supposed, the heat formation resulting from the respiration process probably would cease, not at 45° to 50°, but perhaps at 60° or higher. On this assumption a temperature level might be reached without the cooperation of micro-organisms beyond which purely chemical and physical processes would account for the heat production.

Boekhout and DeVries (*1*) concluded from their investigations of the heating of hay and tobacco that micro-organisms have no part in the phenomenon, but that spontaneous heating of hay is only a chemical oxidation process in which iron occurring naturally in the plant acts as a catalyzer.

Haldane and Makgill (*4*) determined the relations of oxygen absorption and carbon dioxide liberation in samples of hay through which a constant flow of pure air was passed. They confirmed the biological conclusions of Miehe and concluded further that even at temperatures as low as 38° to 41° C. a simple chemical oxidation takes place side by side with the biological oxidation. The chemical oxidation could be distinguished easily from the biological by the fact that the former diminished with lapse of time, and by the ratio of carbon dioxide liberated to the oxygen consumed. This ratio was always considerably below unity in the chemical oxidation and about equal to or greater than unity in the biological oxidation.

At 71° C. there was an enormous fall in the oxidation rate, since all microbial activity was suspended and only the chemical oxidation

[3] Reference is made by number (italic) to Literature Cited, p. 546.

continued. A further rise of temperature to 81° caused a great increase in the chemical oxidation rate, and a similar result occured at 90°. According to Haldane and Makgill, the rapid development of heat in a large mass of damp hay is due almost entirely to the growth and activity of micro-organisms, which can raise the temperature to about 70°, but oxidation occurs in the damp hay even when microbial activity is absent.

Truninger (*8*), after discussing oxygen respiration and intramolecular respiration of plants and the role of bacteriological activity in heat production, suggested the desirability of investigating whether the activity of certain bacteria and molds in the fermenting haystack may produce easily oxidizable substances which might serve as the source of subsequent purely chemical processes.

The theory of the spontaneous heating and ignition of large masses of hay proposed by Browne (*2*) is based upon the preliminary production by micro-organisms under more or less perfect anaerobic conditions of unsaturated, highly unstable, intermediate-fermentation products upon the surfaces of the porous, cellular materials. The duration of existence of these readily oxidized products depends upon the quantity of air that gains access to the fermenting mass of hay and also upon the quantity of moisture present as a reacting medium. If the air has free access these compounds are destroyed almost as soon as formed, with the result that when the vegetative micro-organic life is all destroyed, at 70° to 80° C., there is not a sufficient residue of such easily oxidizable substances to carry the heat production to higher limits. The heat of the microbial life period is probably due in large measure to the oxidation of the same unstable products that participate in raising the temperature above 80°.

LARGE-SCALE EXPERIMENTS

PROCEDURE

The experiments reported here comprise 5 of the 10 spontaneous heating experiments completed in the experimental hay barn on the United States Animal Husbandry Farm at Beltsville, Md. A brief statement regarding each experiment follows. In all the experiments alfalfa hay containing less than 30-percent moisture is referred to as cured alfalfa.

Experiment 1, June 1929

The hay stored in experiment 1 was 14 tons of undercured alfalfa, containing a minimum of 30.69 and a maximum of 43.45 percent moisture, an average of 36.35 percent for seven levels of mow, 1, 3, 6, 7, 8, 11, and 12 feet from the floor of the barn. The hay was stored on June 7 to a height of 12 feet in a corner section of the barn, 16 by 16 feet, leaving the east and south sides of the mow exposed. A maximum temperature of 72° C. was reached on June 15, in the center of the mow. After June 16 the temperature throughout the mow continued to drop until, on August 27, the average of the five highest temperatures was 41°. The final height of the mow was 8 feet at the center.

Experiment 3, July 1930

In experiment 3, as in all others following this, the entire floor space of the barn (26 by 24 feet) was used for storage, except for a narrow alleyway (3 by 24 feet) on the entrance, or east side of the barn, thus

exposing the mow on only one side. The hay used was 55,987 pounds of baled alfalfa, having an average moisture content of 15.23 percent. As the hay was put into the mow, it was torn apart and sprinkled with 40,000 pounds of water, bringing its moisture content up to an average of 50.55 percent. The barn was filled to a height of 15 feet on July 22 to 25. The final settled height of the mow was 13 feet. A temperature of 87.5° C. was reached on July 30 at thermocouple 20, located 6½ feet from the floor, 8 feet directly east of the center of the mow, and 4 feet from the alleyway on the east. On August 20 the maximum temperature, 88°, was recorded at a location 9½ feet from the floor, 8¼ feet from the eastern alleyway, and slightly to the north of the center of the mow. On September 5 several thermocouples still registered as high as 70°.

Experiment 4, June 1931

In experiment 4 the hay was stored on June 2 to 4. Dry timothy containing 10.10-percent moisture was put in up to a height of 4½ feet from the floor; 14,900 pounds of undercured alfalfa, having an average moisture content of 56.10 percent, was put over an area of 10 by 10 feet in a central section of the mow, up to a height of 12½ feet; the undercured section was covered and surrounded to a height of 16½ feet by cured alfalfa having an average moisture content of 28.46 percent. The maximum temperature, 78° C., was recorded on June 15 at thermocouple 25, in the cured alfalfa just above the center of the undercured section. The temperature here had dropped to 71° on June 26, on which date the three next highest temperatures were 68°, 63.5°, and 61.5°. When the hay was removed from the barn, on July 6 to 8, the settled height was 10 feet.

Experiment 5, July 1931

The hay used in experiment 5 was dry timothy, containing 16.08-percent moisture, on the bottom of the mow to a height of 2 feet; cured alfalfa of 19.78-percent moisture content to a height of 6½ feet; undercured alfalfa of 31.34-percent moisture content to a height of 9½ feet; and dry timothy on top of this to a height of 15 feet. The maximum temperature, 56° C., was reached on July 19 to 20, 6 days after storage (July 13 to 16), at thermocouple 18, located at the junction of the cured and undercured alfalfa in the southeast section of the mow. On August 20, when the hay was removed from the barn, the maximum temperature was only 35.5°.

Experiment 9, June 1933

The hay in experiment 9 was stored from June 5 to 13 and removed on October 10. It consisted of cured alfalfa, containing 29.86-percent moisture, on the bottom of the mow to a height of 4¼ feet; undercured alfalfa of 43.17-percent moisture content to a height of 8½ feet over an area of 10 by 10 feet in a central section; and cured alfalfa of 29.86-percent moisture content surrounding and covering the undercured hay to a height of 16½ feet. At the time of removal the hay had settled to a height of 13 feet. The highest temperature, 68° C., was reached on June 13 at thermocouple 20, located 6 feet from the floor, at the exact center of the junction of the cured and undercured hay. From June 14 the temperature throughout the mow fell rapidly until July 5, when thermocouple 20 registered 60°.

APPARATUS FOR GAS SAMPLING

The apparatus used in the collection of the samples of gas is shown in figures 1 and 2. The gas-sampling device (fig. 1, A, B, and C), consists essentially of an aluminum capillary tube (*a*) having a bore

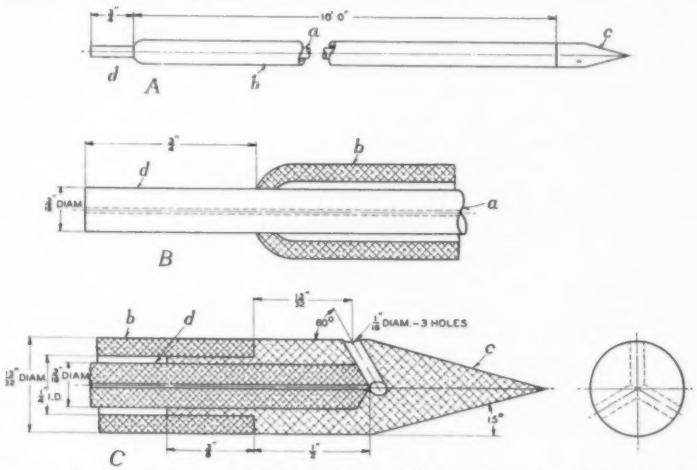

FIGURE 1.—Gas-sampling device: A, complete device; B, end of tube opposite removable tip; C, removable tip.

of approximately 0.02 inch supported by a larger tube (*b*) of the same metal of 13/32-inch diameter. It is through this capillary tube that the sample of gas passes during collection. The length of tube used

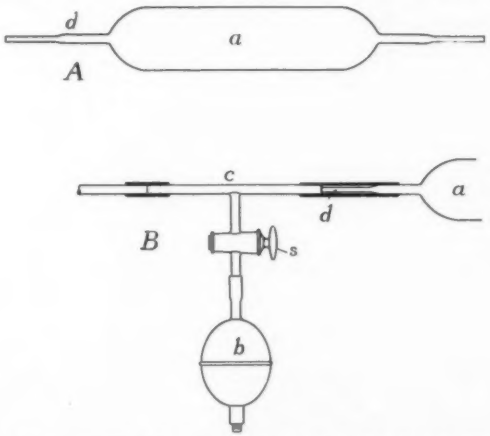

FIGURE 2.—Gas-sampling tube and connections: A, tube; B, tube and connections.

is determined in each case by the distance to which it is desired to penetrate the mow for sampling. Five-, ten-, and twelve-foot lengths have been found satisfactory. In operation, the end of the tube

carrying the removable tip (*c*) is inserted to the desired point in the mow and the other end of the tube (*d*) is connected by rubber tubing through the capillary T-tube (fig. 2, *c*) to one end of the gas-sampling tube (fig. 2, *a*). The glass tube (*a*) has a capacity of approximately 250 cc. Previous to use it is sealed off at one end, highly evacuated, and then sealed off at the other end. The metal capillary and glass connections are then cleared of air by closing the stopcock (*s*), compressing the rubber bulb (*b*) attached to the third arm of the capillary T-tube, and then reopening the stopcock, allowing gas to be drawn into the bulb. Then the stopcock is closed again, and the scratched tip of the gas-sampling tube is broken at (*d*), whereupon the desired sample of gas is drawn by vacuum into the gas-sampling tube. Immediately upon detaching, the broken tip of the gas container is inserted airtight into a wax-sealing material contained in a small metal tube, such as a .32-caliber cartridge. This material is a mixture of beeswax and Venice turpentine in proportions varying with the temperature at which it is to be used. The sample is then ready for analysis. A Burrell precision-model gas-analysis apparatus is admirably adapted for this purpose.

RESULTS

The results of the analysis of the gases collected in the several experiments, together with other data pertaining to the samples, are given in table 1.

TABLE 1.—*Analyses of gas samples collected in large-scale spontaneous heating experiments*

TABLE 1.—*Analyses of gas samples collected in large-scale spontaneous heating experiments*

EXPERIMENT 1, JUNE 1929 [1]

Sample no.	Date of sampling	Period after storage of hay	Location in mow: Height from floor	Location in mow: Distance from north	Location in mow: Distance from south	Location in mow: Distance from east	Location in mow: Distance from west	Nearest thermocouple	Temperature at nearest thermocouple on day of sampling	Previous maximum temperature at same locality: Temperature	Previous maximum temperature at same locality: Date	CO_2 content	O_2 content	O_2 loss (20.86–O_2)	Ratio CO_2 to O_2 loss
		Days	*Feet*	*Feet*	*Feet*	*Feet*	*Feet*		°*C.*	°*C.*		*Percent*	*Percent*	*Percent*	
1	June 10	3	9½	8	8	8	8	29	50.5	45.5	June 9	0.10	20.69	0.17	.....
								25	55.0	63.0	...do.....				
2	...do.....	3	9	0	16	14	2	26	59	60.5	...do.....	.12	20.46	.40	.....
								22	50.5	53.0	June 8				
3	June 13	6	9	1½	14½	11½	4½	26	70.0	71.0	June 12	1.77	18.74	2.12	0.835
								22	58.0	53.0	June 8				
4	...do.....	6	8	11	5	8½	7½	24	65.5	67.0	June 12	4.18	15.87	4.99	.838
								19	68.0	68.5	...do.....				
5	June 21	14	10	3	13	6	10	26	57.0	71.0	...do.....	2.68	17.68	3.18	.843
								22	53.0	58.0	June 11				
6	...do.....	14	9	8	8	8	8	29	51.0	61.5	June 14	2.42	18.28	2.58	.938
								25	60.5	70.5	June 15				
7	June 28	21	5	8	8	7½	8½	17a	60.0	72.0	...do.....	3.94	16.87	3.99	.987
8	...do.....	21	5	8	8	9½	6½	17a	60.0	72.0	...do.....	3.80	16.90	3.96	.960
9	Aug. 27	81	5	8	8	7½	8½	17a	49.0	72.0	...do.....	1.45	19.34	1.52	.954

EXPERIMENT 3, JULY 1930

1	July 28	5	[2] 2½	11½	11½	11	11	.....	.....	.....	.....	1.32	19.42	1.44	0.916
2	...do.....	5	2	11½	11½	5½	16½	9	82.0	79.0	July 27	13.72	5.75	15.11	.908
3	...do.....	5	5	11½	11½	3½	18½	20	86.0	81.0	...do.....	13.46	5.95	14.91	.903
4	...do.....	4	6½	17½	5½	5½	16½	28	82.0	80.0	...do.....	7.53	12.54	8.32	.905
5	Aug. 5	13	2	11½	11½	5½	16½	9	76.0	83.0	July 30	10.35	8.31	12.55	.825
6	...do.....	12	6½	17½	5½	5½	16½	28	77.0	82.0	July 28	5.76	14.22	6.64	.867
9	Aug. 14	21	7½	6½	16½	6½	15½	z	84.5	83.0	Aug. 1	16.60	2.94	17.92	.926
10	...do.....	21	7½	7	16	7	15	y	84.0	83.0	...do.....	5.21	14.95	5.91	.881
11	Sept. 9	47	5¼	11½	11½	11	11	32	69.0	81.0	Aug. 24	11.40	9.43	11.43	.997
12	...do.....	47	4¼	11½	11½	11	11	32	69.0	81.0	...do.....	13.34	7.31	13.55	.984

[1] Sample 1 was taken 1 inch below top of mow. Samples 2, 3, and 4 were taken from hot spots, 1, 1½, and 2 feet below top of mow. Samples 5 and 6 were collected 3 and 4 inches from top of mow.

[2] Above top of stack.

TABLE 1.—*Analyses of gas samples collected in large-scale spontaneous heating experiments*—Continued

Sample no.	Date of sampling	Period after storage of hay	Location in mow					Nearest thermocouple	Temperature at nearest thermocouple on day of sampling	Previous maximum temperature at same locality		CO_2 content	O_2 content	O_2 loss (20.86-O_2)	Ratio CO_2 to O_2 loss
			Height from floor	Distance from north	Distance from south	Distance from east	Distance from west			Temperature	Date				
		Days	*Feet*	*Feet*	*Feet*	*Feet*	*Feet*		°*C.*	°*C.*		*Percent*	*Percent*	*Percent*	
EXPERIMENT 4, JUNE 1931 [3]															
1	June 24	19	9	13	11	11	12	25	72.5	78.0	June 15	2.20	18.33	2.53	0.87
2	do	19	10	10½	13½	17	6	27	56.0	61.0	June 13	1.87	19.01	1.85	1.01
3	do	19	8	12	12	9	14	25	72.5	78.0	June 15	2.63	17.90	2.96	.89
4	do	19	7	14½	9½	13	10	20	71.5	73.5	do	2.21	18.69	2.17	1.02
EXPERIMENT 5, JULY 1931 [4]															
1	Aug. 17	32	4¾	18	6	6½	16½	18	37.0	56.0	July 19	0.66	20.20	0.66	1.00
2	do	32	5½	12	12	9	14	14	32.0	51.0	July 20	.59	20.48	.38	1.56
								20	31.0	52.0	do				
3	do	32	7	12	12	11½	11½	25	30.0	52.0	do	.43	20.77	.09	
								20	31.0	52.0	do				
4	do	32	7	16	8	7	16	18	37.0	56.0	July 19	.78	20.57	.29	2.70
								11	35.5	54.0	July 18				
EXPERIMENT 9, JUNE 1933 [5]															
1	June 21	15	6	13	11	10	13	20	66.5	68.0	June 13	3.20	18.78	2.08	1.54
2	do	15	4	13	11	10	13	y	65.0	67.5	June 10	2.96	18.77	2.09	1.42
3	do	15	4	13	11	8½	14½	14	63.0	64.5	June 13	2.30	19.43	1.43	1.61
4	July 7	31	5	13	11	10	13	20	56.5	68.0	do	3.96	17.91	2.95	1.34
								y	54.0	67.5	June 10				
5	do	31	4	13	11	8½	14½	14	56.0	64.5	June 13	3.11	18.82	2.04	1.52
6	July 19	43	5	13	11	10	13	20	56.0	68.0	do	3.02	17.98	2.88	1.05
								y	51.5	67.5	June 10				
7	Aug. 3	58	4	13	11	8½	14½	14	53.0	64.5	June 13	.91	20.20	.66	1.38
8	do	58	5	13	11	10	13	20	55.0	68.0	do	2.16	19.07	1.79	1.21
								y	53.5	67.5	June 10				

[3] Samples 1, 2, and 3 were taken from areas of cured alfalfa which had become wet from rise of H_2O, from undercured section below. Sample 4 was taken from undercured section.
[4] Sample 1 was collected from junction of cured and undercured alfalfa; sample 2, from center of undercured alfalfa; sample 3, from cured alfalfa; sample 4, from undercured alfalfa.
[5] Sample 1 was collected at the junction of cured and undercured alfalfa; all other samples were from the undercured section.

All the samples in experiments 1, 3, 4, and 5 were analyzed for carbon monoxide, hydrogen, and methane. Carbon monoxide and hydrogen were determined by combustion in the copper oxide tube at 290° to 310° C. (after removal of constituents soluble in caustic potash, fuming sulphuric acid, and pyrogallol), and methane was determined in the slow-combustion pipette. Methane was absent in all the samples, and neither carbon monoxide nor hydrogen was found in the samples taken in experiments 4 and 5. Carbon monoxide and hydrogen were found in only one sample in experiment 3, and the amounts in that were within the limits of experimental error. One sample in experiment 1 appeared to contain approximately 0.1 to 0.2 percent of carbon monoxide, while the contraction of the gas upon combustion over copper oxide in five of these samples indicated a possible hydrogen content of 0.1 to 0.3 percent. In several of the samples in experiments 1 and 3, a considerable quantity of the gas was removable by fuming sulphuric acid. Tests for phosphine and hydrogen sulphide on all samples in experiment 1 and on a large proportion of those in experiment 2 gave negative results. Practically all the samples of gas had a peculiar pungent odor similar to that of aldehydes and esters.

It will be observed that in experiment 1 the samples of gas from sources where temperatures of 68° to 70° C. prevailed at the time of collection, or previously, show an oxygen loss greater than the carbon dioxide formed.

The data for experiment 3 also show definite evidence of excess oxygen consumption. Furthermore, the large quantities of carbon dioxide formed in the heating haymow, combined with the high degree of exhaustion of the available oxygen shown by most of the samples, indicates more than ordinary activity. This indication is borne out by observations made of the physical qualities and appearances of the hay when removed from the mow, namely the preponderance of spoiled, brown and black, acid-charred material.

The activity in experiment 4, where a maximum temperature of 78° C. was observed, is fairly comparable with that in experiment 1, with a maximum of 72°, notwithstanding the fact that the absolute quantities of carbon dioxide in the samples are generally somewhat lower. The conditions of storage and the smaller volume and weight of the wet hay may have permitted a greater dilution of the gases with air from outside sources, which, in spite of the slightly higher and longer prevailing temperatures, would cause a lower carbon dioxide content and a correspondingly lower value for the oxygen consumption.

In experiment 5, the relatively small volume of undercured hay and its comparatively low moisture content appear to account for the low degree of activity, with low carbon dioxide production and lower oxygen loss. The results as a whole may be taken to indicate conditions of the ordinary "sweating" process.

In experiment 9, 68° C. was the maximum temperature observed, and it will be noted that the CO_2:O_2-loss ratio is greater than unity in all the samples.

LABORATORY OXIDATION EXPERIMENTS

PROCEDURE

The purpose of this laboratory investigation was to determine the relative tendency to oxidation of natural undecomposed alfalfa hay and similar alfalfa hay which had undergone spontaneous heating in the mow or had been fermented upon exposure while wet. It was premised that if unsaturated or other easily oxidized substances were present in the decomposed samples, these samples should absorb relatively more oxygen than the undecomposed samples, and would throw light on the question of the production of unsaturated oxidizable substances during spontaneous heating. The difficulty of securing samples of heated hay from the mow without exposing them to the air was recognized at once; hence, in the first large-scale experiment, nitrogen was constantly passed through the boring tube used for removing the samples and the sample containers were filled in an airtight box in which the air had been displaced by nitrogen. Analysis of the protecting gas taken from the sample containers (half-gallon fruit jars) at the time the samples were removed for oxidation tests proved the efficiency of this method. As these samples did not show much absorption of oxygen, the use of protecting gas was abandoned in subsequent experiments but every other precaution was taken to avoid undue exposure.

A temperature of 80° C. was selected for the comparison because of the general agreement among investigators that at this temperature all microbial activity has ceased. To confirm the presumption that such action is absent also in the hour's time of attaining the excluding temperature range, this initial heating was investigated. Experiments were conducted at 90° to show the effect of higher temperature. The possible analogy of such oxidations to the well-established avidity of linseed oil and other unsaturated oils to take up oxygen was not overlooked, and a test on boiled linseed oil impregnating an equal weight of cotton waste was conducted under identical conditions.

APPARATUS

The apparatus in which these experiments were conducted is shown in figure 3. *a* is a Pyrex round-bottom flask having a capacity of 1,054 cc when tightly closed with the three-hole rubber stopper *e* carrying a thermometer and a capillary tube which emerges through the stopper to *f*. A small condenser tube (internal diameter about 4 mm) of the condenser *d* is connected through the third hole of the stopper. This tube is joined, by means of rubber tubing at *g*, to a capillary tube bent as shown, which in turn is connected at *h* to the small end of the inverted separatory funnel *b* of approximately 500-cc capacity joined at the tubulature by rubber tubing to a leveling bulb. Above the point *h* a capillary tube is sealed on at a right angle and connected with a small open mercury manometer *m*. On the other side of the flask *a*, connection is made from *f* by capillary tubing to *k*, where it is joined by rubber tubing to *c*, a cylindrical glass tube having a capacity of about 300 cc, and provided with stopcock *l*. This container also is provided with a leveling bulb. *o* is a cylindrical oil bath in which flask *a* is immersed in heavy mineral oil. This bath is heated from below by means of an electric hot plate *p*, and within the

oil by an electric heater *n*, carrying a stirrer and regulated by means of a bimetallic thermostat *r*. The oil bath has an inside diameter of 33 cm and is well insulated on its sides with a 5-cm thickness of asbestos. By means of the regulator and heater, temperatures can be maintained to within 0.4° C. The total volume of the closed system from stopcock *s* to stopcock *l*, including flask *a*, the condenser, manometer tube, and capillary tubing of 0.5-mm diameter, is 1,060 cc.

In conducting an experiment, the mercury used in *b* and *c* is raised to stopcocks *s* and *l*, respectively, and the stopcocks are closed. A weighed sample is then placed in *a*, the rubber stopper carrying the thermometer, the condenser, and the capillary tube is tightly inserted, and connections are made at *f* and *g* by means of rubber tubing. The temperature of the air and sample and the barometric pressure are noted at the time of closing the system, and the enclosed air volume (1,060 cc) is later corrected for air displaced by the sample. This displacement is calculated from the specific gravity determined experimentally on the dry matter contained in several representative types

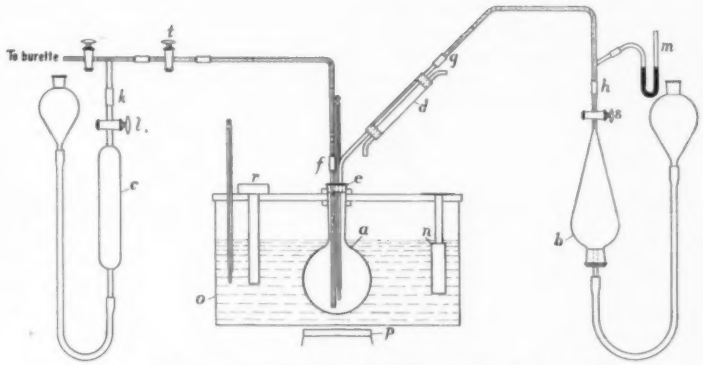

FIGURE 3.—Apparatus for oxidation experiments.

of samples. From the average of these figures, which varied little, a correction value was obtained of 0.7 cc of air displaced per gram of dry matter. This corrected volume is reduced to the arbitrary standard conditions of 25° C and 760 mm pressure, the tension of water vapor being taken into account.

The bath is now heated by the electric hot plate until the temperature has reached to within about 10° of the desired bath temperature; then the heater within the oil is substituted as the source of heat and serves for all subsequent heating. As the temperature of the flask *a* and its contents rises, the excess of expanding air is allowed to collect in container *b*, the stopcock of which has been opened at the start of heating. During this time and throughout the entire experiment, the level of the mercury is kept adjusted to atmospheric pressure as indicated by the manometer levels. If, as in some experiments, container *b* is not sufficient to take care of the air expansion, container *c* also may be used for the purpose. The small condenser *d* serves to condense and avoid loss of moisture in the sample.

When the bath reaches the temperature desired, the temperature in flask *a* containing the hay sample is recorded as the initial temperature for the subsequent 4-hour period of heating at the fixed bath temperature. Temperature readings are recorded at frequent intervals during the heating. The temperature of the sample and air in the flask was found to be always somewhat lower than the bath temperature, in most cases probably owing to the evaporation of water.

At the end of the 4-hour period the bath is cooled as rapidly as possible by means of cold water passed through cooling coils located around the interior walls of the bath. During the cooling some of the previously expanded air collected in *b* is returned to *a* to take care of the contraction in *a*. When the bath temperature has fallen to 50° to 60° C., stopcocks *t* and *l* are opened, and the gas is passed back and forth through *a* from *b* to *c* until the entire volume of gas has become thoroughly mixed. Finally all the gas is removed from *b*, and stopcock *s* is closed. The sampling of gas for analysis is then completed by drawing into *c* the volume required for several analyses. From *c* the gas can be transferred readily through capillary connection into the burette used in the analysis.

The time required to raise the oil bath from room temperature to 80° C. was remarkably uniform in the experiments, the records showing a maximum of 68, a minimum of 52, and an average of 61 minutes. To raise it to 90° required only a few minutes longer. The cooling from 80° to 90° to 50° to 60° required an average of 23 minutes.

RESULTS

The results of the laboratory experiments are recorded in table 2.

TABLE 2.—*Results of laboratory experiments on the oxidation of hay*

UNDERCURED ALFALFA AND SLIGHTLY FERMENTED ALFALFA (BATH TEMPERATURE, 80° C.)

Sample no. and condition	Weight of sample	Moisture content	Dry matter: Weight	Dry matter: Ash	Temperature of sample	Volume of original air (25°, 760 mm) (79.14 percent N_2) (A)	Composition of original air: O_2 (B)	Composition of original air: N_2	Volume final gas based on N_2 of original air (C)	Composition of final gas: CO_2		Composition of final gas: O_2 (D)		Composition of final gas: N_2		Loss of gas [1] (A) – (C)	Available O_2 at maximum temperature	O_2 absorbed (B) – (D)	CO_2 formed	O_2 absorbed per 25 g ash-free dry matter	CO_2 formed per 25 g ash-free dry matter	Ratio CO_2 formed to O_2 absorbed
	Grams	*Pct.*	*Grams*	*Pct.*	*°C.*	*Cc*	*Cc*	*Cc*	*Cc*	*Pct.*	*Cc*	*Pct.*	*Cc*	*Pct.*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	
S(8)	50	46.30	26.85	6.97	75.0–77.4	1,010.60	210.81	799.79	999.86	3.46	34.59	16.55	165.48	79.99	799.79	10.74	107.49	45.33	34.59	45.37	34.62	0.763
S(9)	40	35.90	25.64	7.78	74.0–77.0	971.18	202.59	768.59	965.57	4.82	46.54	15.58	150.44	79.60	768.59	5.61	107.27	52.15	46.54	55.14	49.21	.892
1(10)	40	39.00	24.40	7.42	78.0–78.5	1,002.30	209.08	793.22	982.45	3.72	36.55	15.54	152.67	80.74	793.23	19.85	103.05	56.41	36.55	62.43	40.51	.648
S(9) fermented	40	38.26	24.70	7.78	74.4–77.0	1,031.45	215.16	816.29	1,026.52	5.10	52.35	15.38	157.88	79.52	816.29	4.93	109.14	57.28	52.35	62.87	57.46	.914
S(9) moldy	40	37.10	25.16	7.78	77.0–77.6	990.52	206.62	783.90	987.03	5.83	57.54	14.75	145.59	79.42	783.90	3.49	105.65	61.03	57.54	65.76	61.99	.943
7(4)[2]	50	48.42	25.79	9.89	76.0–77.6	1,011.82	211.06	800.76	995.10	4.48	44.58	15.05	149.76	80.47	800.76	16.72	105.77	61.30	44.58	65.94	47.95	.727
9(4)	50	56.16	21.93	9.89	75.0–77.0	957.28	199.68	757.60	946.88	4.55	43.08	15.44	146.20	80.01	757.60	10.40	105.21	53.48	43.08	67.66	54.50	.806
S(4)	60	69.80	18.12	9.89	75.0–79.5	998.50	208.29	790.21	988.26	4.53	44.77	15.51	153.28	79.96	790.21	10.24	97.29	55.01	44.77	84.22	68.55	.814
6(4) fermented	50	57.78	21.11	9.89	75.0–77.5	964.63	201.22	763.41	946.10	6.45	61.02	12.86	121.67	80.69	763.41	18.53	104.78	79.55	61.02	104.55	80.19	.767

UNDERCURED ALFALFA AFTER SPONTANEOUS HEATING (BATH TEMPERATURE, 80° C.)

A(9)	40	21.80	31.28	10	75.4–76.0	1,032.20	215.32	816.88	1,031.41	0.72	7.42	20.08	207.11	79.20	816.88	0.79	111.57	8.21	7.42	7.29	6.59	0.904
18(1)	40	23.05	30.78	9.08	74.0–77.7	1,015.27	211.79	803.48	1,023.29	1.74	17.81	19.74	202.00	78.52	803.48	+8.02	104.25	9.79	17.81	8.74	15.91	1.819
B2(4)	30	19.01	24.30	14	71.2–73.6	1,026.69	214.16	812.53	1,026.18	0.72	7.39	20.10	206.26	79.18	812.53	.51	119.21	7.90	7.39	9.45	8.84	.935
B3(9)	40	24.27	30.29	10	73.0–77.0	1,005.38	209.72	795.66	1,009.72	1.78	17.97	19.42	196.09	78.80	795.66	+4.34	106.49	13.63	17.97	12.50	16.48	1.318
13(1)	40	24.97	30.01	11.71	74.0–77.0	1,014.77	211.69	803.08	1,020.17	2.02	20.61	19.26	196.48	78.72	803.08	+5.40	107.12	15.21	20.61	14.35	19.45	1.355
B6(4)	30	21.76	23.47	12	73.6–75.0	1,027.14	214.26	812.88	1,027.66	1.35	13.87	19.55	200.91	79.10	812.88	+.52	115.35	13.35	13.87	16.16	16.78	1.039
25(4)	40	39.80	24.08	11.99	75.0–77.0	1,037.12	216.34	820.78	1,034.64	1.65	17.07	19.02	196.79	79.33	820.78	2.48	107.95	19.35	17.07	23.07	20.13	.873
B8(4)	50	43.94	28.03	15.40	76.2–78.8	1,002.22	209.06	793.16	959.89	6.65	63.83	10.72	102.90	82.63	793.16	42.33	101.55	106.16	63.83	111.91	67.29	.601

[1] Increase of gas volume is indicated by plus (+) sign.
[2] Analyzed for combustible constituents.

TABLE 2.—*Results of laboratory experiments on the oxidation of hay*—Continued

UNDERCURED ALFALFA (BATH TEMPERATURE, 90° C.)

Sample no. and condition	Weight of sample	Moisture content	Dry matter		Temperature of sample	Volume of original air (25° C., 760 mm) (79.14 percent N_2) (A)	Composition of original air		Volume final gas based on N_2 of original air (C)	Composition of final gas				Loss of gas: (A)−(C)	Available O_2 at maximum temperature	O_2 absorbed (B)−(D)	CO_2 formed	O_2 absorbed per 25 g ash-free dry matter	CO_2 formed per 25 g ash-free dry matter	Ratio CO_2 formed to O_2 absorbed
			Weight	Ash			O_2 (B)	N_2		CO_2	O_2 (D)	N_2								
	Grams	*Pct.*	*Grams*	*Pct.*	°C.	*Cc*	*Cc*	*Cc*	*Cc*	*Pct.* *Cc*	*Pct.* *Cc*	*Pct.* *Cc*		*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	
B6(4) wetted	25.5	[3]60.00	19.95	12.00	75.0–90.2	996.89	207.96	788.93	1,008.10	2.16	21.78	19.58	197.39	78.26	788.93	+11.21	22.14	181.21	45.85	23.71	57.36	29.66	0.517
18(1)	40	23.05	30.78	9.08	79.8–86.8	999.70	208.54	791.16	1,006.30	3.36	33.80	18.02	181.34	78.62	791.16	+6.60	179.14	1.87	2.53	3.46	4.68	1.352
B6(4)	30	21.76	23.47	12.00	75.0–85.7	1,032.29	215.32	816.88	1,044.61	3.39	35.42	18.41	192.31	78.26	816.88	+12.41	192.72	22.01	75.13	23.01	35.42	27.85	42.88	1.539
B3(9)	30	24.27	30.29	10.00	83.0–86.2	1,020.65	212.91	807.74	1,021.55	3.12	31.87	17.81	181.94	79.07	807.74	+.91	180.97	30.97	72.58	30.97	31.87	28.40	29.22	1.029
25(4)	40	39.80	24.08	11.99	78.0–88.5	1,049.40	218.91	830.49	1,054.99	4.06	42.83	17.22	181.67	78.72	830.49	+5.59	67.04	37.24	42.83	43.93	60.53	1.150
S(8)	40	46.30	21.48	6.97	80.0–88.0	1,022.52	213.30	809.22	1,030.19	5.22	53.77	16.25	167.20	78.55	809.22	+7.67	62.47	46.10	53.77	57.67	67.27	1.166
B8(4) [2]	40	43.94	22.42	15.40	85.0–89.0	1,035.78	216.06	819.72	1,022.98	6.56	67.10	13.31	136.16	80.13	819.72	+12.80	59.71	79.90	67.10	105.31	88.44	.840
MISCELLANEOUS																						
S(8) [2]	40	46.30	21.48	6.97	[4]45.0	1,035.02	215.91	819.11	1,012.88	2.34	23.71	16.79	170.06	80.87	819.11		22.14	181.21	45.85	23.71	57.36	29.66	0.517
S(4) dry	15	0	9.89	85.5–90.5	1,054.99	220.07	834.89	1,055.62	.24	2.53	20.67	218.20	79.09	834.89		+.66	179.14	1.87	2.53	3.46	4.68	1.352
S(4) dry+H_2O	20	50	10.00	85.0–90.4	1,036.31	216.17	820.14	1,040.13	3.47	36.10	17.66	183.89	78.85	820.14		+3.82	54.36	32.28	36.10	89.56	100.16	1.118
S(4) [2] dry	15	0	9.89	100.0–100.6	1,067.12	222.60	844.52	1,064.56	.75	7.98	19.92	212.06	79.33	844.52		2.56	175.43	10.54	7.98	19.50	14.76	.757
Boiled linseed oil	10	0	10	.....	77–81.2	1,065.83	222.23	843.50	888.08	.49	4.35	4.53	40.23	[5]94.98	843.50		177.75	184.69	182.10	4.35	455.25	10.88	.024
7(4)a	50	48.42	25.79	9.89	75.4	1,000.12	208.62	791.50	998.74	1.07	10.59	18.96	167.65	79.97	791.50		10.38	113.24	20.97	10.59	22.56	11.40	.505
7(4)b [2]	50	48.42	25.79	9.89	76.0–77.6	1,011.82	211.06	800.76	1,005.10	4.48	44.58	15.05	149.76	80.47	800.76		16.72	165.77	61.30	44.58	65.94	47.95	.727
1(10)a	40	39.00	24.40	7.42	77.5	1,006.2	209.89	796.31	1,004.04	.81	8.13	19.88	189.00	79.31	796.31		2.16	104.36	10.29	8.13	11.39	9.00	.790
1(10)b	40	39.00	24.40	7.42	78–78.5	1,002.30	209.05	793.22	992.45	3.72	36.55	15.54	152.67	80.74	793.23		19.85	163.05	86.41	36.55	62.43	40.51	.648
1(10)c	40	39.00	24.40	7.42	76.6–77.6	995.7	207.75	788.00	974.17	8.54	92.94	9.57	93.23	80.89	788.00		21.53	104.13	114.47	92.94	126.68	102.86	.812
1(10)d	40	39.00	24.40	7.42	76.6–77.6	1,006.8	210.02	796.78	994.24	8.53	84.81	11.33	112.65	80.14	796.78		12.56	106.10	97.37	84.81	107.76	93.86	.871

[3] By addition of water.

[4] For 78 hours.

[5] Includes approximately 0.48 percent of combustible gas.

In this table the samples of hay are designated by a combination of numbers or of letters and numbers. The letter S indicates that the sample was the standard laboratory sample drawn before storage and corresponding to the hay stored in wire-cloth containers (or "baskets") in the respective large-scale experiments. The letter B indicates that the sample was from one of such baskets after storage. The number in parentheses is the number of the large-scale experiment. In the sixth column, which shows the temperature range during the 4-hour heating period, the initial temperature of the period is given as the lower limit, but the temperature prevailing during practically all that period was very close to the maximum temperature recorded. For the purpose of more accurate comparison the oxygen absorbed and carbon dioxide formed are computed for equal amounts (25 g) of moisture and ash-free sample, and with the exception of section 4 the results are arranged in the order of increasing oxygen absorption. Available O_2 at maximum temperature is that portion of the original oxygen remaining in the oxidation flask when the air had been expanded by heating to the maximum temperature of the experiment. All volumes given in the table are for 25° C. and 760 mm.

The values given in the table for oxygen absorbed and carbon dioxide formed are not corrected for the relative solubility of these gases in the water of the hay samples at the temperature and pressure of sampling. Such corrections would not exceed 1 cc for carbon dioxide and 0.15 cc for oxygen in the hay sample with the highest water content, and in no case would the tabulated results be materially affected by these corrections.

Some of the samples of gas were analyzed for combustible constituents, with negative results.

In the case of boiled linseed oil, however, the combustion over copper oxide resulted in a contraction of volume equivalent to 0.16 percent of hydrogen and a production of carbon dioxide equal to 0.32 percent of carbon monoxide. Attention is called to a recent experiment by Haldane and Makgill (*5*) in which carbon monoxide occurred in the gas resulting from the oxidation of boiled linseed oil at 18° C. According to Haldane and Makgill, small quantities of carbon monoxide are formed also in the oxidation of wet hay at 40°, when bacterial activity is entirely excluded. Under the influence of bacteria, however, hydrogen is formed.

In table 2, section 1, S(8) was a sample of undercured alfalfa taken in September 1932 from a field in the vicinity of Beltsville, Md. Sample S(9) was undercured alfalfa from another field near Beltsville, collected in June 1933. Sample S(9) fermented was a sample of the same hay after exposure in the laboratory to warm moist air for 3 weeks, while S(9) moldy had been exposed to room temperature for 11 days in a loosely closed jar. Sample 1(10) was undercured alfalfa collected on June 12, 1934, from a field also in the vicinity of Beltsville. S(4), 7(4), 9(4), and 6(4) were undercured alfalfa from the dairy husbandry farm at Beltsville. Sample 6(4) was slightly sour when tested.

The effect of moisture is indicated in samples 7(4), 9(4), and S(4) of hay from the same field. The oxidation increases with the moisture content. In samples S(9) fermented, which had undergone microbial changes, and S(9) moldy, in which considerable mold had formed, the oxidation was greater than in S(9). For apparently the same reason

the oxidation was greater in the soured sample 6(4) than in samples 7(4), 9(4), or S(4). The ratio of CO_2 formed to oxygen absorbed is less than unity in all experiments.

In table 2, section 2, samples A(9) and B3(9) were from the same hay as S(9) (sec. 1) but had undergone spontaneous heating. These samples were collected from a location in the mow (table 1, experiment 9) where a maximum temperature of 68° C. was reached 7 days after storage and where a temperature between 68° and 60° prevailed for 28 days afterward. Sample B3(9) had undergone a decomposition loss in dry organic matter of nearly 14 percent. Samples 18(1) and 13(1) were similar, having been collected in experiment 1 from localities in which an average temperature of 68° had prevailed for several days. Samples B2(4), B6(4), 25(4), and B8(4) were the result of the heating during storage of undercured hay S(4), 7(4), and 9(4) (sec. 1). Samples B2(4) and B6(4) had been stored in a section of cured alfalfa where the temperature for about 25 days averaged 55° and 58°, respectively; B2(4) had lost 22 percent and B6(4) 19 percent of dry organic matter. Sample 25(4) was collected from a location in the originally undercured alfalfa where the temperature had ranged from 68.5° to 60° for 30 days. Sample B8(4) had undergone a heating of 73.5° to 63° for 26 days in a wire basket located in the undercured alfalfa section of the mow. This sample had lost a little less than 11 percent of dry organic matter during storage, but the metal basket had become badly corroded, and the sample was contaminated with copper and nickel as indicated by the high ash content and proved by the analysis of the ash.

With the exception of this contaminated sample the results for all the experiments in section 2 show very low oxygen absorption and carbon dioxide formation as compared with those in section 1. It is possible that this is due largely to the comparatively lower moisture content of the samples in section 2, although such an effect is not indicated in sample 25(4), which has the highest moisture content but only a slightly greater oxygen absorption and carbon-dioxide formation. Apparently the lower results must be ascribed to the losses of organic matter during storage and to the previous destruction of any unsaturated substances that might have been formed in the course of spontaneous heating.

The experiments recorded in section 3 of table 2 were carried out at temperatures about 10° higher than those in sections 1 and 2. The effect of this higher temperature is shown in the increased oxidation in 5 of the 6 cases, excluding sample B6(4), to which water had been added, and in an increase in the proportion of carbon dioxide formed to oxygen absorbed in 4 of the same 6 cases. In sample B8(4) the oxygen available for oxidation at the maximum temperature had been greatly decreased from the corresponding value shown in section 2 by the expansion accompanying the elevation of temperature.

The experiment with sample S(8) (sec. 4) was designed to show the behavior of undercured alfalfa when subjected to oxidation under conditions believed to be favorable for microbial action. Accordingly a sample was kept at a temperature of 45° C. for 78 hours in the same apparatus and according to the identical procedure followed in the other experiments at higher temperatures. In this experiment the oxygen absorbed was greater, but the carbon dioxide formed was less

than in the experiment with the same hay at 75° to 77.4° (sec. 1). The ratio of CO_2 formed to O_2 absorbed was consequently lower than at the higher temperature.

The alfalfa of previous experiments, sample S(4), completely dried and ground, was heated as shown in table 2, section 4. There was very little oxidation, but the carbon dioxide exceeded the oxygen loss. To another sample of the same dry and ground hay an equal weight of water was added, and the oxidation was observed. The effect of this wetting is quite apparent in the enormously increased oxidation. Here again the carbon dioxide formed exceeded the oxygen absorbed.

A third sample of this dry, ground hay heated around 100° C. showed a greater oxidation than the same dry hay at 85.5° to 90.5°, with a reversal of the ratio of carbon dioxide formed to oxygen consumed. Presumably some destructive distillation was to be expected here, and if it occurred it appears to have been accompanied by the formation of products that increased the relative absorption of oxygen.

The results of the experiment with boiled linseed oil show the character and comparative degree of the oxidation of unsaturated oil and very probably furnish the clue to the nature of the chemical oxidations of hay, insofar as these may involve the oxidation of unsaturated substances formed as the result of heating.

The character and extent of the oxidation which occurs in the interval of approximately 1 hour required to bring the temperature up to the temperature at which the 4-hour period of heating begins are shown in the case of samples 7(4)a and 1(10)a. It is evident that this initial oxidation is considerable, but of essentially the same character as shown by the other experiments with the same hay, in which the heating was continued for the usual 4 hours, samples 7(4)b and 1(10)b. Furthermore, if the values for oxygen consumed and carbon dioxide formed in samples 7(4)a and 1(10)a are subtracted from the corresponding values for samples 7(4)b and 1(10)b, it will be seen that the ratios of carbon dioxide formed to oxygen consumed in the 4 hours in which the oxidation was confined to the maximum temperature are not materially affected.

Experiments were made to determine the effect of heating more than the arbitary 4 hours. Sample 1(10)c was heated for 20 hours. It was then heated for an additional 20 hours with a fresh supply of air, sample 1(10)d. The results show a progressive oxidation, incomplete but of approximately the same character.

DISCUSSION

The results of the laboratory oxidation experiments (table 2) considered in relation to the results of the analysis of the gases collected in the large-scale experiments (table 1) throw considerable light on the question of chemical activity in the heating haymow. In the first place, the laboratory experiments at temperatures between 77° and 80° C., where chemical oxidation occurred in natural undercured alfalfa without the intervention of micro-organisms, show that at these temperatures chemical action is accompanied by an oxygen consumption in excess of the carbon dioxide formed, and that this oxidation is progressive and of the same character regardless of whether it is limited to 4, 20, or 40 hours (table 2, secs. 1 and 4).

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On the other hand, the experiments with undercured hay which had already suffered oxidation in the mow show that at these same temperatures chemical oxidation may result in the production of carbon dioxide in excess of the oxygen consumption (table 2, sec. 2). Furthermore, the results of heating at approximately 90° C. show that at this temperature there is increased chemical activity accompanied by an increase in the proportion of carbon dioxide formed to the oxygen absorbed (table 2, sec. 3). It is very probable that in these samples some of the oxygen combined chemically in the earlier stages of chemical oxidation may be liberated as CO_2 in the later stages, in which case the carbon dioxide produced may exceed the oxygen absorbed.

It may be concluded, therefore, in harmony with the previous conclusions of Haldane and Makgill (*4*), that a consumption of oxygen greater than the liberation of carbon dioxide is an evidence of chemical oxidation. But it does not follow that all chemical activity is limited to those instances in which the carbon dioxide liberated is less than the oxygen consumed.

If, therefore, an oxygen consumption in excess of carbon dioxide production is evidence of chemical oxidation, in order to determine the occurrence of chemical action in the presence of biological activity in the spontaneous heating of hay, it is necessary to assume that the production of carbon dioxide in amounts equal to or greater than the oxygen consumed is characteristic of biological action. This assumption appears to be justified by the observations of Haldane and Makgill referred to above, and it is in agreement with the results of extensive investigations of the mechanism of plant respiration and micro-organic activity, at least where the resultant gaseous product is carbon dioxide alone.

Upon the basis of this distinction between chemical and biological activity, the results of the investigation of the gases collected in the large-scale spontaneous-heating experiments show that, along with the respiration processes of the living plant cell and the activity of micro-organisms, chemical oxidation occurred where a temperature range of 68° to 70° C. prevailed (table 1, experiment 1). For the production of the temperature required for the initiation of this chemical action, in the absence of other evidence, the operation of biological agencies must be assumed. Upon the same basis, chemical activity is established also for temperatures between 72° and 78° (experiment 4), and this is still more positively indicated in experiment 3, where temperatures between 76° and 88° prevailed for several weeks.

The results of experiments 5 (maximum temperature, 56° C.) and 9 (maximum temperature, 68°) do not show chemical oxidation, but they do not exclude the possibility of such oxidation as may have occurred along with the greater activity of micro-organisms.

From the evidence thus far presented the conclusion is justified that chemical oxidations take place in the heating haymow at a temperature as low as 68° C. The evidence of chemical activity is not so satisfactory for temperatures much below this. However, the laboratory experiment on sample S(8) (table 2, sec. 4) may be interpreted as showing chemical oxidation combined with microbial action. As already stated, this experiment was expected to show the behavior of undercured alfalfa subjected to oxidation under

conditions believed to be favorable for microbial action. In this experiment the hay was kept at 45° for 78 hours, and the same procedure was followed as in the other experiments at higher temperatures. Here the relation of carbon dioxide formation and oxygen consumption was found to be of the same order and character as in the same alfalfa at approximately 77°. The $CO_2:O_2$ loss ratio was far below unity. If this experiment may be accepted as showing chemical action at 45°, it would be reasonable to conclude that, throughout the whole range of temperature usually ascribed to the action of biological agencies, chemical oxidations also occur, simultaneously or alternately. The results obtained in the investigation of the oxidation occurring in the interval of approximately 1 hour required to raise the temperature of the hay samples to approximately 75° (samples 7(4)a and 1(10)a, table 2, sec. 4) lend support to this conclusion. This initial oxidation was considerable and of essentially the same character as the subsequent oxidation up to a maximum of approximately 80°.

The existence of chemical activity in a mass of hay in which the temperature has risen from any cause whatever to the neighborhood of 80° C. is further confirmed by the fact that in the laboratory experiments such activity was produced by heat supplied from external sources. Similar activity obviously must be inferred in the case of the heating mass of hay at a corresponding temperature.

The discussion of the results of this investigation would not be complete without a consideration of the significance of an oxygen consumption in excess of the carbon dioxide liberated in the laboratory oxidation experiments. Perhaps the occurrence of small quantities of unsaturated substances in the hay may have some influence. Again, it might be assumed that there is an unequal absorption or adsorption by the heated hay of oxygen and carbon dioxide, which would affect the ratio of carbon dioxide formed to oxygen consumed. But under the conditions of experimentation it does not appear at all probable that the hay was converted into such a condition that it would have much adsorptive property. It is far more probable that the excess oxygen consumption was mainly owing to incomplete oxidations in which there was no corresponding carbon-dioxide formation, or to the production, under the influence of heat and chemical action, of unsaturated substances which were destroyed at once by oxidation.

Unfortunately, because of the nature of the experiments, it was not possible to obtain direct proof of the presence of unsaturated substances in the products of reaction. The same difficulty was experienced in the effort to show their presence in the samples of undercured hay which had undergone spontaneous heating in the mow prior to the laboratory tests (table 2, sec. 2). The comparatively low oxidation results in the case of these samples indicate that a partial or complete oxidation of any easily oxidized unsaturated products that might have been formed there had already occurred in the mow.

The desirability of further investigations to determine whether or not unsaturated substances are readily formed in hay, either by purely chemical processes or by the action of micro-organisms, is quite obvious. And if such investigations should demonstrate that under the influence of heat supplied from external sources, with the exclusion

of microbial activity, unsaturated products are formed, the interesting question would arise whether chemical activity in the heating haymow may not be due in part to the production by heat of unsaturated substances susceptible to oxidation. However, it would be necessary to assume that the heat production in the mow is due mainly to biological agencies, and consequently the distinction between the formation of unsaturated substances by microbial agencies and their formation as the result of the heat of microbial activity would be one only of proximate or ultimate cause.

SUMMARY

The results of an investigation of the gases collected during the spontaneous heating of alfalfa hay and comparative results of oxidation experiments in the laboratory are presented.

Apparatus for the collection of gases from the heating haymow and a laboratory oxidation apparatus are described.

The results of the investigation indicate that, along with the operation of biological agencies in the heating haymow, there occurs a purely chemical oxidation, evidenced by a loss of oxygen considerably in excess of the carbon dioxide formed. This chemical oxidation is more marked beyond the temperature range usually ascribed to the activity of micro-organisms.

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THE CONSTRUCTION OF NORMAL-YIELD AND STAND TABLES FOR EVEN-AGED TIMBER STANDS[1]

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INTRODUCTION

A normal-yield table is a tabulated statement of yields to be expected from well-stocked, even-aged stands of a given timber type, at successive ages, for the range of site qualities characteristic of the type. Normal-stand tables are frequency distributions of tree stems by diameter classes within stands of given age and site quality.

All of the timber-yield tables published in the United States during the last 10 years have been based, either entirely or partially, upon the Bruce method (*1*)[2] or Reineke's modification of it (*7*). The accompanying stand tables have usually been based upon one of three principles of construction: (1) Correlation of percentiles of frequency with average diameter of stand (*2*, *8*); (2) graphic fitting of either the normal curve of error or some logarithmic transformation of the normal curve to actual frequency distributions (*1*, *7*); or (3) mathematical fitting of the Gram-Charlier series to the actual distributions after harmonizing calculated parameters with average diameter (*4*, *5*, *9*, *10*).

It is the purpose of this paper to describe (1) a general method of constructing yield tables for the entire stand that is free from the inherent rigidity of the Bruce-Reineke method, and (2) a method of constructing stand tables that is more objective than the purely graphic and less laborious in calculation than the Gram-Charlier series.

YIELD TABLES

The data upon which yield and stand tables are based consist of measurements of sample plots in the desired timber type. The sample plots selected should be in even-aged stands and large enough to contain 100 to 300 trees. The objective is to enclose a comparatively complete crown canopy by excluding the larger openings which follow accident or failure of reproduction and at the same time to include the ground area equivalent to that used by the enclosed timber. After surveying plot boundaries in order to determine plot area, the following measurements are taken: (1) Diameter breast high[3] of every tree by species and crown class; (2) heights of enough trees (usually 15 to 25) to permit the reliable estimate of average height of each diameter class; and (3) age counts, taken with an increment borer on several trees, to establish plot age.

From these data, the yield and the stem distribution of each plot are calculated on an acre basis. Yield is expressed in several ways, such as number of trees, basal area, average diameter, and volume in cubic and board feet. The relation of yield to age and site quality

[1] Received for publication Apr. 30, 1935; issued December 1935.
[2] Reference is made by number (italic) to Literature Cited, p. 564.
[3] Four and a half feet above the ground; abbreviation, d. b. h.

is then worked out. For the western white pine type, as an example, Haig (*3*) constructed tables for the following expressions of yield:

A. For the entire stand:
   - Number of trees per acre.
   - Average diameter breast high.
   - Basal area per acre.
   - Cubic-foot volume inside bark per acre.

B. For the trees 6.6 inches d. b. h. and larger:
   - Number of trees per acre.
   - Average diameter breast high.
   - Basal area per acre.
   - Board-foot volume per acre, according to the International log rule.

C. For the trees 7.6 inches d. b. h. and larger:
   - Board-foot volume per acre, according to the Scribner log rule.

D. For the trees 12.6 inches d. b. h. and larger:
   - Number of trees per acre.
   - Average diameter breast high.
   - Basal area per acre.
   - Board-foot volume per acre according to the International log rule.
   - Board-foot volume per acre according to the Scribner log rule.

E. For the dominant stand:
   - Average diameter breast high.
   - Cubic-foot volume per acre.
   - Height of average dominant (dominant and codominant) western white pine.

In Haig's investigation, as in practically all such studies, the direct relation of yield to age and site quality was worked out for the basic variables of the entire stand only, yields of the partial stand (with the exception of the height of the average dominant western white pine) being derived therefrom.

These derived curves of yield have proved workable and satisfactory. But the curves of yield of the entire stand in terms of age and site quality are forced into too rigid a mold. The nature of this rigidity will be shown and a method of analysis that entirely overcomes it described. The data consist of measurements of 99 fully stocked, even-aged sample plots of red gum (*Liquidambar styraciflua* L.) measured by R. K. Winters of the Southern Forest Experiment Station.

THE DETERMINATION OF SITE INDEX

The quantitative measure of site quality, known as site index, is the height of the average dominant (usually including the average codominant) tree at a given age, called reference age, for which 50 years is commonly taken, as in the present discussion. A necessary condition for the correct determination of site index is that it be intrinsically independent of plot age; for then the proportion of the sample plots whose site indexes are better—or poorer—than any given site index is the same, in the long run, at one age as at another, and, conversely, site index may be identified by associating it with the probability of occurrence. For example, the site qualities that are the most productive 10 percent of the area in a timber type should be represented by 10 percent of the sample plots in each age class whose height of average dominant—or dominant and codominant—tree is greatest.

The condition stated above may be fulfilled in field sampling if, while trying for approximately equal numbers of plots in each age class, conscious effort is made to secure random distribution of the sample plots with respect to site quality. It then follows that the curve of height on age (shown in fig. 1, *A*, for the red gum data) is

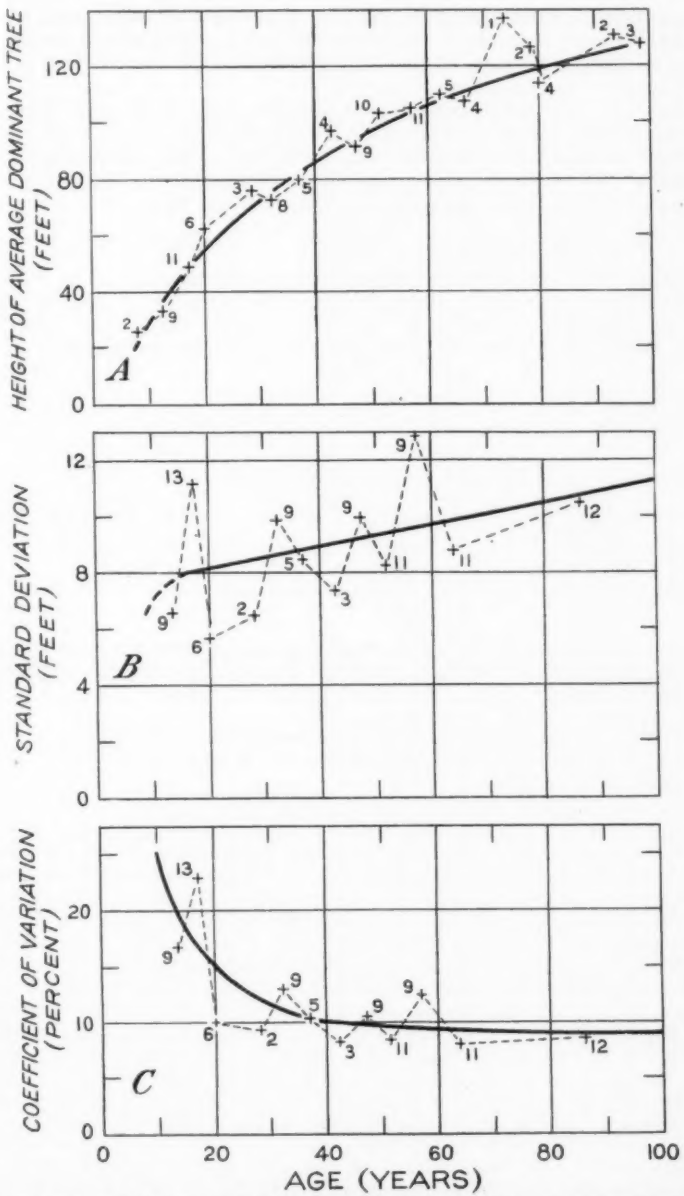

FIGURE 1.—Steps in the determination of site index: *A*, Relation of height of average dominant tree to age; since age and site index are independent of each other, this curve is also that of site index 98 feet at 50 years. *B*, Relation of the standard deviation of height of average dominant tree to age. *C*, Relation of the coefficient of variation to age.

not only the gross regression but also a partial regression, that is, it represents the relation of height to age for a constant site index as well as for the average of the site indexes, in this case 98 feet at 50 years.

The curves of height on age for other site indexes are next needed. In all of the recent yield studies in this country these have been defined as curves of the same form as that of average site index but differing therefrom by a constant ratio at all ages; for example, the curve of height of dominant stand on age for the 80-foot site index of red gum would be defined as $\left(\frac{80-98}{98}\right)$ or -0.184; that is, 18.4 percent less than the curve values of figure 1, *A*, at any age.

Now if site index thus defined holds true in nature, it follows that the coefficient of variation of height is independent of age. Otherwise there will not be the same proportion of the sample plots of a

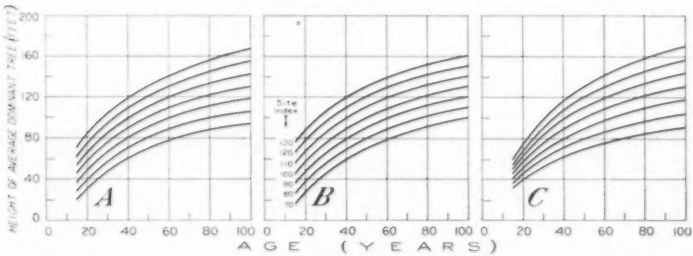

FIGURE 2.—The relation of height of average dominant tree to age and site index: *A*, As determined from the relation of the standard deviation or of the coefficient of variation to age; *B*, on the assumption that the standard deviation is independent of age; *C*, on the assumption that the coefficient of variation is independent of age.

given site class in all ages. This is an important hypothesis, and one that may be tested in each investigation. A method of making the test will be illustrated.

The distributions of residuals from the curve of the average site index of figure 1, *A*, are collected in table 1 by selected age classes. At the bottom of the table are given the standard deviations in feet, and the coefficients of variation; the latter are the standard deviations expressed as percentages of the curve value of the mean. The relation of these two constants of distribution to age is shown in figure 1, *B* and *C*. The three curves of figure 1 permit of ready cross checking, because any value of the standard-deviation curve divided by the corresponding average must equal the coefficient of variation. Since, according to the figure, the coefficient of variation is not independent of age in the early life of the stand, the definition upon which site index has generally been determined in yield studies during the past decade does not hold for the red gum data; that is, the ratio of height of dominant stand of a given site index to that of the average site index is not constant, but depends, rather, upon the age of the stand.[4]

[4] Roy A. Chapman, of the Southern Forest Experiment Station staff, tested this hypothesis for 3 of the southern pines for which yield tables had been published; for 2 of them it did not hold.

TABLE 1.—*Frequency distribution of the plot residuals from figure 1, A, by selected age classes*

Deviation of height of average dominant and codominant tree from curved height for average site index (feet)	Plots in age class indicated											
	8–14 years	15–19 years	20–24 years	25–29 years	30–34 years	35–39 years	40–44 years	45–49 years	50–54 years	55–59 years	60–69 years	70+ years
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
+19.50 to +22.49		1										1
+16.50 to +19.49										1		1
+13.50 to +16.49									1			
+10.50 to +13.49	1						1		2	1	1	
+7.50 to +10.49		1		1					1	1	1	
+4.50 to +7.49	1	2	6		3	1	1	2	1	1		
+1.50 to +4.49	2	2		1	1			1	1	2	2	3
−1.50 to +1.49		1			1	1		2	2		3	4
−4.50 to −1.49	1	1				2	1		1	1	1	2
−7.50 to −4.49	3				2							
−10.50 to −7.49		1							1		2	
−13.50 to −10.49	1				1			2	1			
−16.50 to −13.49		3						1				
−19.50 to −16.49		1				1		1				
−22.50 to −19.49										2	1	
−25.50 to −22.49					1							1
Total	9	13	6	2	9	5	3	9	11	9	11	12
Standard deviation[1] feet	6.6	11.2	5.7	6.5	9.9	8.5	7.4	10.0	8.3	12.9	8.8	10.5
Curved height at average age of class feet	39.5	49.2	56.1	70.0	76.2	82.6	89.8	95.2	99.1	104.4	109.3	123.1
Coefficient of variation percent	16.7	22.8	10.2	9.3	13.0	10.3	8.2	10.5	8.4	12.4	8.1	8.5

[1] Computed from deviations measured to the nearest foot. The data are grouped into 3-foot classes for conciseness.

Fortunately, however, the curves of height of dominant stand which result from such varying ratios may be easily deduced from figure 1. Again taking the 80-foot site index as an illustration, the curve for the height of dominant stand, as determined above, is 18.4 percent less than the curve values of figure 1, *A*, but this is at the reference age of 50 only. It should, however, be this same number of units of the coefficient of variation at all ages, so that the probability of occurrence of a given site index would be the same at all ages. Since the coefficient of variation (as read from figure 1, *C*) is 9.5 percent at 50 years, site index 80 becomes $\frac{-18.4}{9.5}$ or −1.94 units measured from the average curve. And 1.94 times the curve value of the coefficient of variation at any given age is the percentage reduction to be applied to the average curve to give the curve height for site index 80 feet at that age. The curves for other site indexes are calculated in a similar manner. Several are shown in figure 2, *A*.

Site index, then, may be assigned to each plot by plotting the height of its average dominant tree over its age on this graph and interpolating between the nearest curves.

In practice it is perhaps simpler to base the site-index curves upon the standard deviation rather than upon the coefficient of variation. As site index is independent of age, though the standard deviation of dominant height may or may not be independent of age, site index may be defined as a deviation in standard units from the curve of dominant height on age. The absolute value of a standard unit at

any age for red gum is given in figure 1, *B*. For the reference age of 50 years it is 9.3 feet. Hence the 80-foot site index curve is $\left(\frac{80-98}{9.3}\right)$ $=-1.94$ standard units measured from the curve of figure 1, *A*. At 100 years of age, for instance, the height of dominant stand for site index 80 feet is $130+(-1.94\times11.3)=108$ feet. This process suggests a general expression of height of dominant stand in terms of age and site index. We have (1) the expression

$$H=f_1(A)$$

where H is height of dominant stand for average site index, expressed as a function f_1 of age A (fig. 1, *A*); (2) the standard deviation σ_H of the height of dominant stand as a function of age A, that is,

$$\sigma_H=f_2(A)$$

where f_2 is such a function as defined in figure 1, *B*. From these two expressions and from the definition of site index as given above,

$$H=f_1(A)+f_2(A)\left[\frac{S-f_1(A_R)}{f_2(A_R)}\right]$$

in which H is height of dominant stand at age A for site index S, and A_R is the reference age which determines the numerical expression of site index. This is a general expression of the joint effect of two independent variables upon a dependent variable when the correlation between the independents is zero.

If the standard deviation had been found to be independent of age, a series of site-index curves, such as those of figure 2, *B*, would have resulted, in which each is a constant difference from any other. On the other hand, had the coefficient of variation been independent of age, the result would have been a series of site-index curves, each a constant ratio of any other (fig. 2, *C*). Since, however, both the standard deviation and the coefficient of variation are dependent upon age, the best estimate, as shown in figure 2, *A*, is intermediate between the others.

THE CONSTRUCTION OF YIELD TABLES FOR THE ENTIRE STAND

The same general method may be used for the expression of entire stand yield in terms of age and site index. Since age and site index are independent of each other, the gross regression of yield on age is also the partial regression for the average of all site indexes present. For a particular site index, however, the regression of yield on age is (in the general case) at a level which differs from the gross regression by a certain number of units of the standard deviation of yield around the gross regression. How the number of such standard units corresponding to each site index is defined may be illustrated through the analysis of yield of red gum in cubic feet and in number of trees.

In figure 3 is shown the relation to age of the gross regression of volume, the standard deviation of volume, and the coefficient of variation of volume. The last-named is primarily for cross checking with the other two. The plotted points were computed in the same way as and the curves are analogous to those of figure 1.

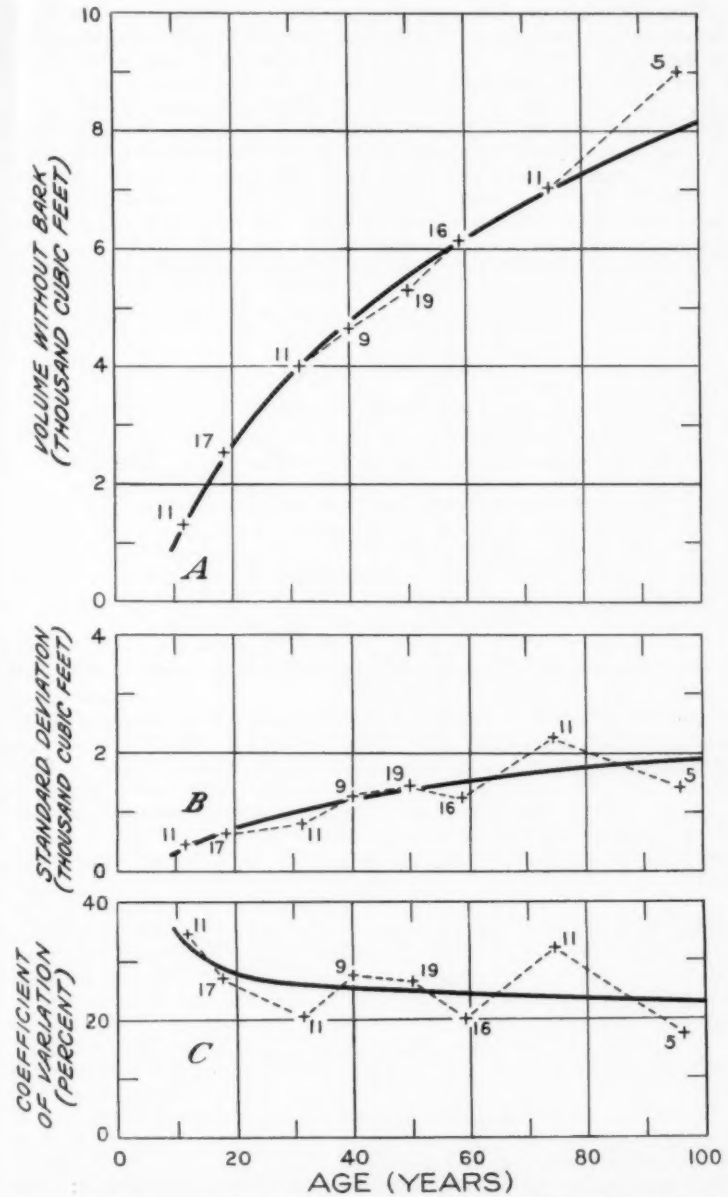

FIGURE 3.—Steps in the correlation of volume with age and site index: *A*, Relation of volume per acre to age for the average of the site indexes; *B*, relation of the standard deviation of volume to age; C, relation of the coefficient of variation of volume to age.

The sample-plot data are next sorted according to site-index classes and entered on a form such as table 2, which shows a partial listing of the data for red gum, site index class 70–74 feet. In column 5 are given the estimated volumes of the plots according to plot age, taken from figure 3, *A*. The residuals are given in column 6. In column 7 are the estimated standard deviations according to age as read from figure 3, *B*, and in column 8 the ratio of the residual to the standard deviation. For each site-index class the average site index and the average of the corresponding standard units are then computed. These standard units are then plotted on site index, and a freehand curve fitted to the points (fig. 4, *A*), the curve defining the deviation of the volume curve of any site index from the

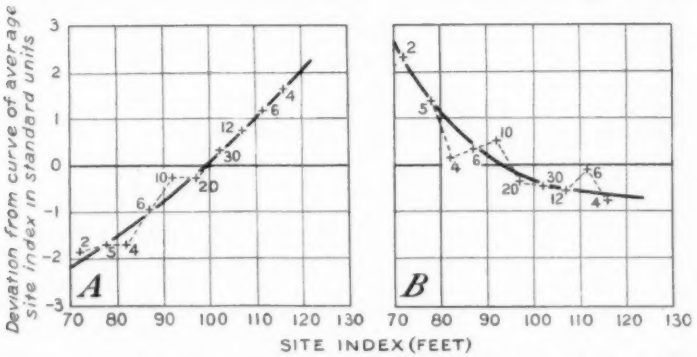

FIGURE 4.—The partial regression of yield (in units of the standard deviation at any age) on site index for *A*, volume in cubic feet, and *B*, number of trees.

volume curve for the average of the site indexes, in standard units of volume at a particular age. It is converted to units of volume by multiplying by the standard deviation for the age.

TABLE 2.—*Form of compilation and calculations preliminary to the determination of the net regression curves of volume in cubic feet on age*

Site-index class (feet)	Site index	Age	Volume per acre			Standard deviation at indicated age	Actual minus curved ÷ standard deviation
			Actual	Curved (for the average site index)	Actual minus curved		
	Feet	*Years*	*M cu. ft.*	*M cu. ft.*	*M cu. ft.*	*M cu. ft.*	*Standard units*
70–74	70	31	1.18	3.95	−2.77	1.02	−2.71
	74	56	3.37	5.92	−2.55	1.46	−1.75
Sum	144						−4.46
Average	72						−2.23

To summarize, three relationships have been established: (1) The expression of volume for the average of the site indexes, in terms of age (fig. 3, *A*) represented by

$$V = f_1(A)$$

in which V is volume, f_1 is its expression in terms of age (A) for the average of site indexes; (2) the standard deviation of volume in terms of age (fig. 3, B), or

$$\sigma_V = f_2(A)$$

in which σ_V is the standard deviation of volume, and f_2 is its expression in terms of age (A); and (3) from figure 4, A—

$$\frac{v}{\sigma_V} = f_3(S)$$

in which v is the deviation of the volume for a given site index from f_1 (A), σ_V has the same meaning as above, and f_3 is the expression of the ratio in terms of site index (S). The volume for any age and site index may therefore be expressed as follows:

$$V = f_1(A) + \{f_2(A) \cdot f_3(S)\}$$

In figures 5 and 4, B, are presented the set of curves needed to express number of trees per acre in terms of age and site index. It is preferable, in the case of this variable, to plot both the average number of trees and the standard deviation on logarithmic scale, as the same relative accuracy is thus assured at all ages.

As stated above, the methods now in use for arriving at the yields of the partial stands have proven workable and satisfactory. These methods are based upon the relation of one of the following values of the partial stand to the average diameter breast high of the entire stand: (1) The ratio of the partial stand yield to the entire stand yield, such as the ratio of the basal area of the trees 6.6 inches d. b. h. and larger to the basal area of the entire stand, or the ratio of board-foot volume in the trees 12.6 inches d. b. h. and larger to the cubic-foot volume of the entire stand; (2) the difference between the partial stand yield and the corresponding yield of the entire stand, such as the difference between the average diameter breast high of the trees more than 12.5 inches and the average diameter breast high of the entire stand.

The yields for the partial stand are calculated by applying the proper ratio or difference to the particular yield of the entire stand which had served at the base of the ratio or difference, by reference to the average diameter breast high of the entire stand.

While this method is subject to a theoretical imperfection in that the ratios or differences as related to the average diameter breast high of the entire stand have not been proven to be completely independent of age and site quality, it has two important practical advantages: (1) Proper weights may be readily assigned to ratios which are correlated with only a single variable; (2) the correlation of ratios, or differences, with average diameter breast high serves to prevent absurdities which may follow the fitting of yield curves independent of one another to age and site quality.[5]

[5] It is obvious, for instance, that the average diameter breast high of the trees 6.6 inches and larger cannot be less than the average diameter breast high of the entire stand; that the ratio of the basal area of a partial stand to the basal area of the entire stand cannot exceed 1.

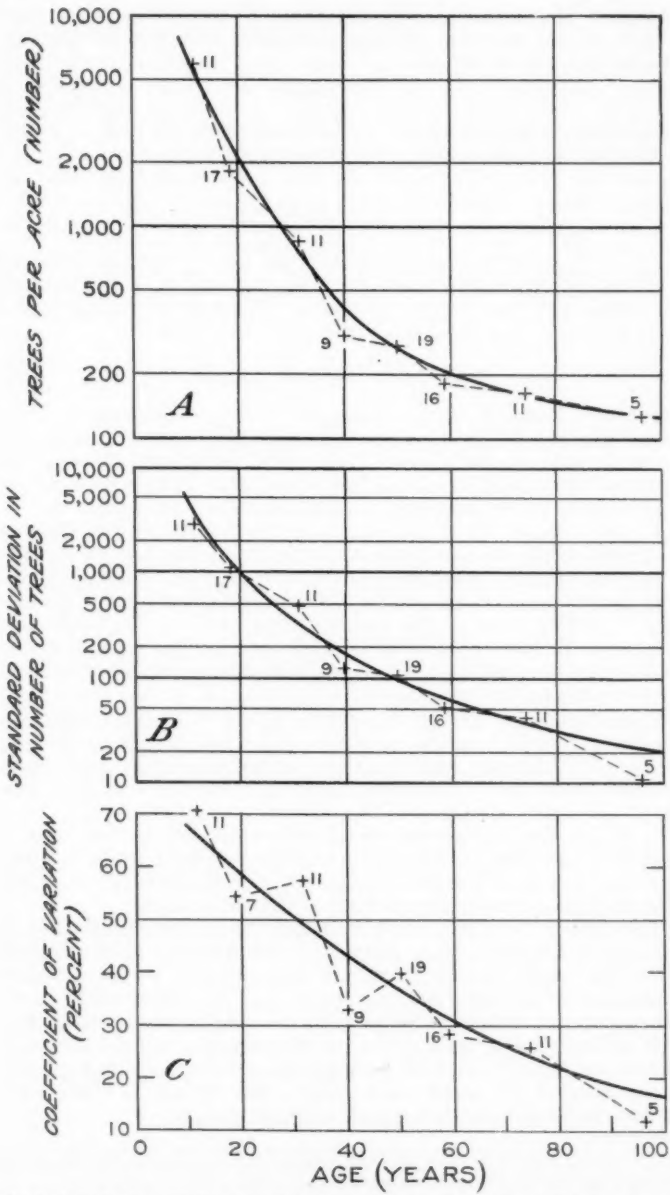

FIGURE 5.—Steps in the correlation of number of trees with age and site index: *A*, Relation of number of trees per acre to age for the average of the site indexes; *B*, relation of the standard deviation of the number of trees to age; *C*, relation of the coefficient of variation to age.

STAND TABLES DEFINED BY THE PEARL-REED POPULATION GROWTH CURVE

The value of an objective method of stand-table construction is apparent. It should be flexible enough to maintain the inherent variation of timber stands, but it should not necessitate laborious computations in application. The search for such a method led to the Pearl-Reed population growth curve (*6*). This curve is of the form

$$y=c+\frac{k}{1+me^{f(x)}} \tag{1}$$

where y is the population in a restricted geographical area, c is the lower asymptote or the population at the beginning of a cultural epoch, $(k+c)$ is the upper limiting asymptote of population, m is an

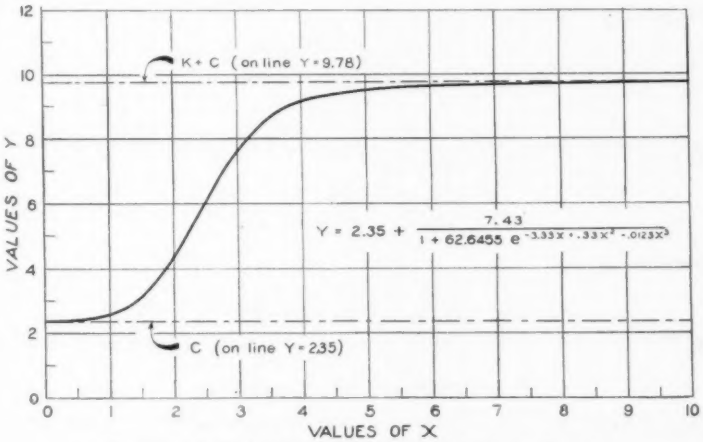

FIGURE 6.—An illustration of the Pearl-Reed population growth curve.

arbitrary constant, e is the base of the Naperian logarithms, x is the date, and $f(x)$ is of the form $b_1x+b_2x^2+- - -+b_nx^n$. Figure 6 is an example of this type of curve.

A special case occurs when $c=o$ and $k=100$. If, under these conditions, y is defined as the number of trees in an even-aged timber stand whose diameters are less than a given limit x, expressed in percentage of the total number of trees in the stand; then the curve of equation (1) closely resembles the cumulative frequency curve of a typical forest-stand diameter distribution (fig. 7).

In an analysis of a curve of this type it is convenient to use the calculus method. A reader not interested in the theory may pass over the subsequent discussion as far as equation (2) without loss of continuity.

The curves of figure 7 approach the ordinates $y=0$ and $y=100$ very slowly; hence these ordinates may be considered asymptotes to the curves. This suggests the condition—

$$\frac{dy}{dx}=(100-y)y\ f(x)$$

for which $\frac{dy}{dx}$ vanishes at $y=0$ and at $y=100$. To facilitate the integration, the right-hand member may be divided by -100. This operation does not change the values of y when $\frac{dy}{dx}=0$. Accordingly,

$$\frac{dy}{dx}=\frac{(100-y)y}{-100}f(x).$$

Separating variables, we have—

$$\frac{-100dy}{(100-y)y}=f(x)dx.$$

Adding and subtracting $\frac{ydy}{(100-y)y}$, this is reduced to—

$$\frac{-dy}{100-y}-\frac{dy}{y}=f(x)dx.$$

Integrating—

$$\int\frac{-dy}{100-y}-\int\frac{dy}{y}=\int f(x)dx+a$$

or

$$\log\left[\frac{100-y}{y}\right]=f'(x)+a.$$

If we define

$$f'(x)=b_1x+b_2x^2+b_3x^3+\text{- - - -}+b_nx^n$$

then

$$\log\left[\frac{100-y}{y}\right]=a+b_1x+b_2x^2+\text{- - - -}+b_nx^n. \qquad (2)$$

The curve is most easily fitted to data in the form of equation (2). Its exponential form is—

$$\frac{100-y}{y}=e^{a+b_1x+b_2x^2+\cdots+b_nx^n}$$

or

$$y=\frac{100}{1+e^{a+b_1x+b_2x^2+\cdots+b_nx^n}} \qquad (3)$$

and this is identical with the Pearl-Reed curve when $c=0$, $k=100$, and $m=e^a$.

In order to determine the number of terms in x necessary to fit equation (2) satisfactorily to even-aged stands as encountered,

coordinates were read from a Pearson type III curve, the β_1 and β_2 coefficients of which were 1.73 and 4.96 respectively; and the following equations fitted successively:

$$\log\left\{\frac{100-y}{y}\right\}=a+bx$$

$$\log\left\{\frac{100-y}{y}\right\}=a+b_1x+b_2x^2$$

$$\log\left\{\frac{100-y}{y}\right\}=a+b_1x+b_2x^2+b_3x^3$$

The first two proved inadequate but the third followed the data closely. This is deemed sufficient reason to apply the last form to the red gum distributions.

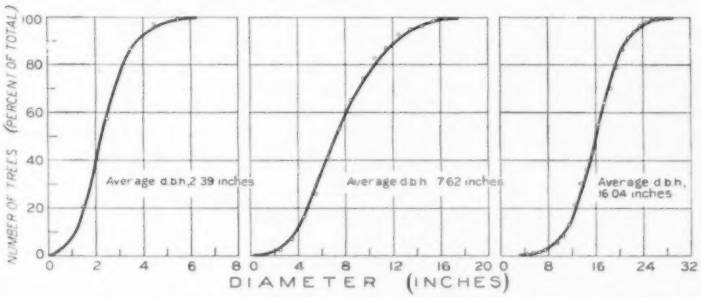

FIGURE 7.—Comparison of actual with calculated cumulative frequencies in stands of small (*A*), medium (*B*), and large (*C*) timber.

APPLICATION TO INDIVIDUAL STANDS

The plots were therefore sorted according to average diameter (by basal area) into groups with ½-inch class intervals. A combined stand tally for each group was then computed, showing the frequency of trees in each 1-inch diameter class from which the arithmetic average of the diameters was calculated. The frequencies of successive diameters, starting with the lowest, were then accumulated, and the cumulative values expressed as percentages of the total frequency. Table 3 shows the form of this summary, a line for each group.

TABLE 3.—*Form of summarizing field data on forest stands*

Average stand diameter class by basal area (inches)	Arithmetic average diameter breast high	Plots	Cumulative frequency percentages (*y*) for diameter class limits (upper), inches (*x*)							
			1.5	2.5	3.5	4.5	5.5	6.5	7.5	8.5
	Inches	*Number*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
2.5–2.9	2.39	5	20.6	57.4	86.8	97.0	99.0	99.6	100	----
3.0–3.4	2.93	3	10.8	46.0	72.0	87.1	95.0	98.1	99.5	100

Defining y as the cumulative frequency to the upper limit of any diameter class, and x as the corresponding upper limit itself, a curve of the form of equation (2) in which—

$$\log\left\{\frac{100-y}{y}\right\}=a+b_1x+b_2x^2+b_3x^3$$

was fitted to the data of each group by the method of least squares, the constants a, b_1, b_2, and b_3 being derived from the solution. In this work the y values were given unit weight and the x values were assumed to be without error.

The actual data of each group and the derived curve were then plotted on the same graph. Three of these comparisons are shown in figure 7. After completing this work for each of the 27 groups, the constants a, b_1, b_2, and b_3 were plotted over the arithmetic average diameter breast high of the group as in figure 8.

ADJUSTMENT OF THE CONSTANTS

The distribution constants plotted in figure 8 should be adjusted in such a way that they progress smoothly with increase in average diameter breast high and also that they be consistent with one another. To effect the required harmonization a method of successive approximation was adopted which permits of the adjustment of two of the constants at a time, through the following relationships:

$$\left.\begin{aligned} na+b_1\Sigma x+b_2\Sigma x^2+b_3\Sigma x^3&=\Sigma\log\left(\frac{100-y}{y}\right)\\ a+b_1M_d+b_2M_d{}^2+b_3M_d{}^3&=0\end{aligned}\right\} \qquad (4)$$

in which n is the number of diameter classes in an actual distribution and M_d is the median diameter breast high of the computed curve. The first equation is simply one of summation over all diameters of an actual distribution. The second equation, based upon the definition of the median, is simply that $y=50$ when $x=$median diameter breast high; that is—

$$\log\left(\frac{100-y}{y}\right)=\log\left(\frac{100-50}{50}\right)=\log 1=0$$

at median diameter breast high. Accordingly, the median diameter was calculated from each curve. The relation of the median diameter to the arithmetic average diameter itself is shown in figure 9. By means of this relation the median diameter breast high for the second of equations (4) is determined.

Since the data of figure 8 define trends of the constants b_2 and b_3 that are obviously more definite than those of a and b_1, freehand curves representing second estimates of the former two constants were drawn as shown. The curve values of each group were then substituted for b_2 and b_3 in equations (4), and the corresponding medians taken from figure 9, as stated above. The simultaneous solution of equations (4) for each group thus affords second estimates of the constants a and b_1 which have well-defined trends and considerably less variation than the first estimates; these were used in drawing the trend curves shown in the upper half of figure 8.

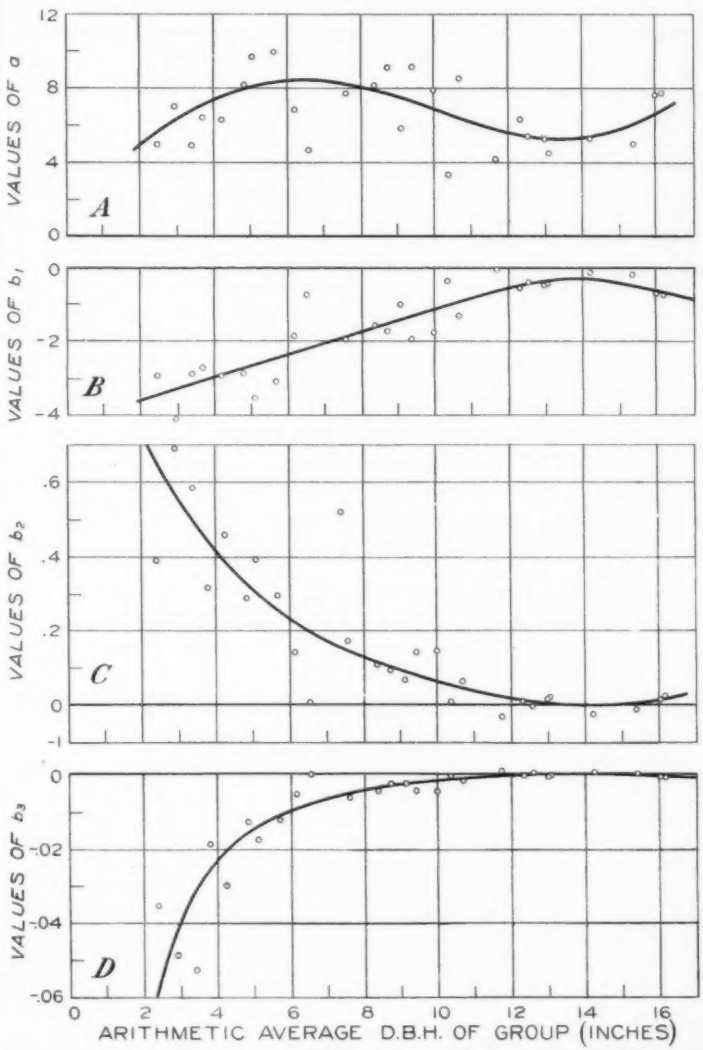

FIGURE 8.—Steps in the adjustment of cumulative frequency constants. The plotted points, called first estimates, were calculated for each group. Freehand curves through b_3 and b_2 (*C*, *D*), called second estimates of these constants, were used to calculate second estimates of a and b_1 (*A*, *B*); through the latter freehand curves were drawn, and these are compared with the first estimates of a and b_1 (*A*, *B*).

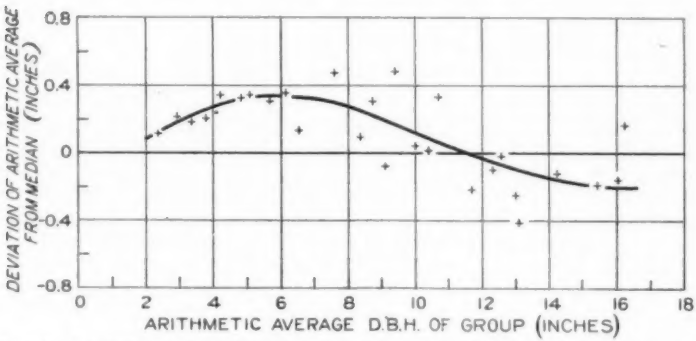

FIGURE 9.—The deviation of the arithmetic average diameter breast high from median diameter breast high as related to the arithmetic average diameter of each group.

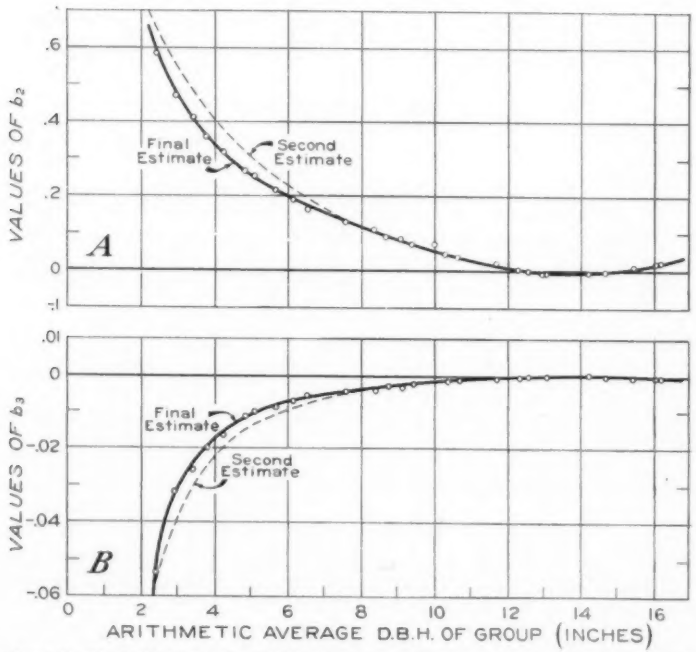

FIGURE 10.—Final adjustment of cumulative frequency constants b_2 and b_3, at *A* and *B* respectively.

As a check, the new curve estimates of a and b_1 were substituted in equations (4), and new values of b_2 and b_3 calculated. In figure 10 these are compared graphically with the previous estimates. Slight changes were made as indicated.

In order to retain three figures for the numerical values of b_2 and b_3, the curves of figure 10 were redrawn on semilogarithmic paper, with breaks, of course, as b_2 becomes negative and b_3 approaches zero. These final adjusted constants permit the calculation of such cumulative stand tables as may be desired, by substituting them in equation (2) or (3). One for each inch of average diameter breast high by basal area is presented in figure 11. Since the constants are expressed in terms of the arithmetic average diameter of the 27 original groups (figs. 8 and 10), a subsidiary curve of the latter on average diameter by basal area was first constructed, and from this, the arithmetic average diameter corresponding to each whole inch of average diameter by basal area was read, and the associated constants taken from figures 9 and 10 as redrawn on logarithmic scale.

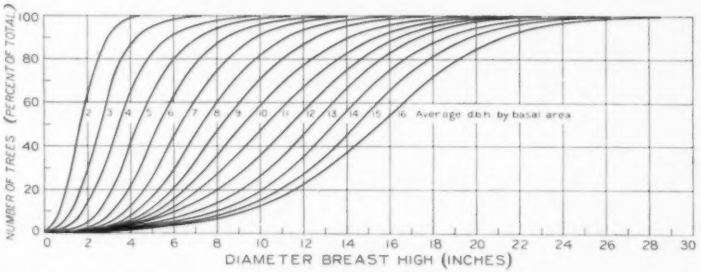
NUMBER OF TREES (PERCENT OF TOTAL)
100
80
60
40
20
0
2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 Average dbh. by basal area
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30
DIAMETER BREAST HIGH (INCHES)

FIGURE 11.—Cumulative frequency percentages in number of trees by diameter-breast-high classes for red gum.

SUMMARY

A method of constructing normal-yield tables is described which does not presuppose, as the Bruce-Reineke method does, that the coefficient of variation of yield of the entire stand is the same at all ages. It is shown, using normal stands of red gum as examples, that the coefficient of variation for height of dominant stand, for volume, and for number of trees is dependent upon age of stand.

The basis of the method is that the relation of the standard deviation, or the coefficient of variation of yield, to stand age determines the form of the growth curve of any site index from the growth curve of the average site index.

Stand tables are constructed by application of the Pearl-Reed population growth curve to cumulative frequency distributions of red gum by diameter-breast-high classes.

A method of harmonizing the curves through adjustment of the descriptive constants two at a time, by successive approximation, is described. The work is less laborious than the use of the Gram-Charlier series and is more objective than the strictly graphic methods of constructing stand tables.

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PHYSIOLOGICAL FACTORS INFLUENCING THE RATE OF EGG FORMATION IN THE DOMESTIC HEN [1]

By D. C. WARREN, *poultry geneticist*, and H. M. SCOTT, *poultry physiologist, Kansas Agricultural Experiment Station*

INTRODUCTION

It is a well-established fact that a bird's annual egg record is conditioned by a number of more or less independent factors. Some of these factors are age at sexual maturity, pauses in production due to broodiness or other inherent tendencies, persistency, and intensity (rate of production).

One of the most important members of the group of factors is rate of production. Hays [2] has presented some evidence that this trait is inherited. The usual measures of intensity or rate of production have been percentage of egg production for a given period or the mean clutch size. Hays and Sanborn [3] have shown that either may be used as a measure of intensity. Considerable attention has been given to the hereditary but very little to the physiological aspects of intensity. It is the purpose of this paper to consider the physiological basis of intensity of egg production. A more adequate knowledge of these features should aid greatly in the genetic approach to the somewhat complicated problem of heredity of rate of egg production.

EXPERIMENTAL FINDINGS

RELATION OF PRODUCTION RATE TO INTERVAL AND CLUTCH

This study is concerned primarily with those features which influence the rate of egg formation. It is a well-recognized fact that individual hens have a characteristic time interval between eggs which is maintained with fair uniformity. The daily egg record is characterized by a succession of groups of consecutive eggs of more or less uniform number separated by a day's pause. The series of eggs on successive days has usually been referred to as a clutch. The rate of production is dependent largely upon the size of the clutch or in other words, the frequency with which the days of nonproduction occur.

Accurate timing of egg laying of individual birds reveals the fact that the length of the interval between successive eggs is an important factor in determining clutch size. The first egg of a clutch is usually laid in the morning and since the interval is ordinarily more than 24 hours the successive eggs are produced somewhat later each day. Thus a bird with a 26-hour interval may lay at 8 a. m., 10 a. m., 12 noon, 2 p. m., 4 p. m., and then miss a day's production. Then the clutch will be repeated after a day's rest. Birds with longer intervals will have smaller clutches. This relationship between interval length and clutch size has been well established by Atwood. [4]

[1] Received for publication June 1, 1935; issued December 1935. Contribution no. 88 from the Department of Poultry Husbandry, Kansas Agricultural Experiment Station.

[2] HAYS, F. A. INBREEDING OF RHODE ISLAND RED FOWL WITH SPECIAL REFERENCE TO WINTER EGG PRODUCTION (PRELIMINARY REPORT). Amer. Nat. 58: 43-59. 1924.

[3] ——— and SANBORN, R. INTENSITY OR RATE OF LAYING IN RELATION TO FECUNDITY. Mass. Agr. Expt. Sta. Tech. Bull. 11, pp. [180]-194. 1927.

[4] ATWOOD, H. A STUDY OF THE TIME FACTOR IN EGG PRODUCTION. W. Va. Agr. Exp. Sta. Bul. 223, 11 pp. 1929.

It is known that there is some relationship between egg production and daylight and darkness since hens do not lay at night and the onset of darkness in some cases appears to be the factor responsible for the termination of the clutch. Previous investigators have believed that the egg which, due to the characteristic interval length, would have been laid in the latter part of the afternoon or at night had been formed normally but, because of the onset of darkness, was not laid at the end of the usual interval. The usual interpretation was that the egg was fully formed at the end of the normal interval but withheld by the hen until the next morning, thus becoming the first egg of the new clutch. Thus if a bird laid at 4 o'clock yesterday and had an interval length of 28 hours she would be due to lay at 8 o'clock tonight. It was thought that today's egg would be fully formed at 8 o'clock but because of darkness would be held until the next morning. If this were the correct interpretation, the egg following a day's pause (the first egg of the clutch) would be in the oviduct much longer than other eggs. Such eggs have been referred to in the literature as held eggs.

It is now known that there is no withholding of the first egg of the clutch and that the formation time is the same regardless of clutch position. The missing of a day between clutches is due to a delay in ovulation instead of the withholding in the uterus of the fully formed egg. This fact was established by bihourly examinations of a number of hens during several days. The position and stage of formation of each egg was determined by handling the hens individually. Figure 1 brings out the fact that the eggs following a pause require no more time for formation than do those laid consecutively. The record shown in figure 1 was obtained under normal lighting conditions.

TIMING EGG FORMATION

In addition to studies from autopsies, time records were secured [5] on five anesthetized hens wherein it was possible to follow the formation of the egg from time of ovulation to its entry into the uterus. From these studies no differences in rate of passage through the oviduct could be detected in hens differing in interval length. Although the number of birds was small this was interpreted to indicate that differences in interval length probably may be accounted for largely by the time spent by the egg in the uterine region of the oviduct.

To secure additional data on the time spent by the egg in different parts of the oviduct of hens varying in interval length, observations were made on two groups of hens. The hens were White Leghorns, Rhode Island Reds, and a few first-generation hybrids. They were kept in individual hen batteries to expedite frequent examination. Two series of 40 hens each were observed for periods of 2 weeks each. The hens were examined at 2-hour intervals from 7 a. m. until 9 p. m. and egg-laying records were taken hourly throughout the day. At each 2-hour interval the position of the egg was determined by probing the cloacal opening. Data were taken on both the position

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—— and SCOTT, H. M. THE TIME FACTOR IN EGG FORMATION. Poultry Sci. 14: 195–207. 1935.

	7 AM	8 AM	9 AM	10 AM	11 AM	12 AM	1 PM	2 PM	3 PM	4 PM	5 PM	7 PM	9 PM
JAN. 7	M		MP		MP		MP		MP		SS	SS	H
8	H	X	O		O		M		M		MP	MP	H
9	H		H		H		H	X	O		O	O	O
10	O		O		M		MP		MP		SS	H	H
11	H	X	O		O		O		M		MP	MP	H
12	H		H		O		⊠		O		O	O	O
13	O		M		M		MP		MP		SS	H	H
14	H		⊠		O		O		M		MP	MP	H
15	H		H		⊠		O		O		M	M	MP
16	H		H		H		H		H		⊠	O	O
17	O		O		M		MP		MP		MP	H	H
18	H		H	X	O		O		M		MP	MP	SS
19	H		H		H		H	X	O		O	O	O
20	O		O		M		MP		MP		MP	MP	H

TIME OF EXAMINATION

FIGURE 1.—Record sheet of bird no. 34 for a 2-week experimental period in which each egg was traced during formation. The hen was examined at the hours indicated and the stage of egg formation recorded. The letters and symbols record the following stages: M, Membranous egg; MP, membranous egg well plumped, SS, soft shell; H, hard shell; X, egg laid since last examination; ⊗, egg laid since last examination and no evidence of the succeeding egg; O, no evidence of egg in oviduct. Thus on January 13, no evidence of an egg was found at 7 o'clock but a membraneous one was found at 9. The egg became plump at 1 o'clock, was soft shelled at 5, and hard shelled at 7. It was hard shelled the next morning at 7 and was laid between the 8- and 9-o'clock examination. The succeeding egg was not found in the oviduct at the 9-o'clock examination on January 14.

of the egg and the stage of formation. The presence of the egg could be detected first at about the time it entered the isthmus. At this early stage the egg could be located only by probing into the large intestine and exploring the region near the dorsal abdominal wall. Experience gained in following a few eggs made it possible to make relatively accurate determinations of position. Records were taken as to the plumpness and degree of shell formation of the egg.

The positions and stages of development utilized in following the egg in the process of formation were: Time of laying of previous egg, first detection of the egg (approximate time of entering the isthmus), plumping of egg after about 2 hours in the uterus, and the beginning of shell deposition. By these observations a comparison could be made of the rate of passage through the oviducts of hens differing widely in length of interval.

A sample record sheet for one hen subjected to normal lighting during the 2-week examination period is given in figure 1. From record sheets such as this, summaries were made (table 1) of the data secured for 2 weeks on the 80 birds under observation. In a preliminary experiment the hens were handled hourly throughout the day, but it was found that such frequent disturbance caused most of the birds to stop laying. The interval between handlings was increased to 2 hours and most of the hens remained in production under these conditions. It was believed that the birds ceased to lay because of the frequent disturbance rather than because of any injury resulting from the exploration of the abdominal cavity.

In the presentation of the data in table 1 an attempt has been made to account for differences in interval length in terms of time spent in different parts of the oviduct.

TABLE 1.—*Mean time spent in different parts of the oviduct by eggs requiring various periods for formation*

Item	Time required for interval lengths [1] of—					
	25	26	27	28	29	30
Time from laying of previous egg to entrance of isthmus by next egg	*Hours* 4.3	*Hours* 4.6	*Hours* 4.2	*Hours* 4.7	*Hours* 5.2	*Hours* 5.3
Time spent in uterus	18.0	18.4	19.9	19.8	20.8	21.6
Time from first indication of shell until egg is laid	13.8	14.7	15.6	16.4	17.0	17.9
Eggs laid	*Number* 9	*Number* 25	*Number* 20	*Number* 42	*Number* 44	*Number* 21

[1] Much of the 5-hour difference in interval length observed here is accounted for in a lengthening of the absolute time spent in the uterus.

FORMATION STAGES AND PERIODS STUDIED

The timed observations made it possible to establish four rather definite stages in egg formation. By using these stages as boundaries it was possible to divide the process of formation into two periods. The stages which the records revealed were: (1) Time of laying of previous egg; (2) first detection of the forming egg about the time it entered the isthmus; (3) the plumping of the egg which occurred about 2 hours after entering the uterus; and (4) the laying of the egg being traced.

The first period included the time between laying and ovulation and also included roughly the time spent in the magnum (albumen-

secreting section). If the egg is the first one of a new clutch this period also includes the extended interval responsible for the interruption in production and the establishment of clutches. Therefore, with the first egg of the clutch, the period intervening between the previous laying and the detection of the succeeding egg has been used as a measure of the delay in ovulation, since the time spent by the egg in the magnum before discovery constitutes only a small part of the whole. When no delay in ovulation occurred, the time between expulsion of the previous egg and ovulation of the next one was so small that the period was considered primarily as a measure of the time spent in the magnum.

The second period was the one between the egg's attainment of a plump stage and its expulsion. This constituted roughly the time spent in the uterus or the period in which shell deposition occurred. It is realized that some of the limits of these periods have been somewhat inaccurately established by the methods of observation utilized but they do offer an avenue of approach to the problem of physiological factors influencing rate of production which would otherwise not be available. Since a large enough number of eggs have been traced to make it possible to express comparisons as means the errors of observation probably have been largely compensated.

INTERVAL AND TIME SPENT IN DIFFERENT REGIONS OF OVIDUCT

In table 1 the data have been segregated on the basis of the interval required for egg formation. The term "interval" is used in this paper to apply to the time ensuing between the laying of successive eggs of the clutch. Thus the interval is a fairly accurate measure of the time required for egg formation. Observations on first eggs of the clutch have been excluded from these data because no accurate knowledge of the time spent in the oviduct could be secured. Due to the delay in ovulation occurring in the case of these eggs the time of the previous laying is of no value in calculating the time required for egg formation. For those eggs following the first of the clutch there intervenes only a few minutes between laying of the one egg and the ovulation of the next. Therefore, in eggs other than the first one of the clutch the time spent in the oviduct and the interval length are practically identical. The data have been so interpreted in calculating the time spent in the various regions of the oviduct in eggs varying in interval length.

The range of period required for egg formation (interval length) in the 161 eggs traced, was from 25 to 30 hours. Many of the eggs produced were not included in this total. All eggs following a pause, and any others where data were incomplete were excluded. Some birds stopped laying during the experiment while others produced many one-egg clutches which were of no value. By calculating the mean time spent in the various parts of the oviduct by the eggs showing variation in interval length, one should be able to determine in what part of the oviduct the extra time is spent by the eggs with long intervals. This should indicate how high- and low-intensity birds differ in egg formation. Intensity of egg production is conditioned by two factors, the interval length and the length of the pause between clutches. In the following discussion those factors responsible for variations in interval length are considered first.

REGIONAL DISTRIBUTION OF VARIATIONS IN INTERVAL LENGTH

It is seen that the extreme range of variations (table 1) in time from laying of previous egg to the entrance of the next egg into the isthmus will account for only a small part of the absolute difference in interval length encountered. The variation in time recorded between laying and the reaching of the isthmus by the next egg was only 1 hour (4.3 to 5.3 hours) while the variation in interval length was 5 hours (25 to 30 hours). There remains the question whether the variation in time required to reach the isthmus has any significance in relation to interval length. In the previous work by the authors [6] the time required for passage of the egg through the magnum in anesthetized birds was very uniform. In the earlier studies there was found to be much more variability in the time between laying and ovulation. The means given in the first line in table 1 include the time between laying of the one egg and the ovulation of the next as well as the time required to traverse the length of the magnum. There was no consistent agreement between the increases in length of the interval and the length of the period between the previous laying and the entry of the next egg into the isthmus. There was, however, a difference of 1 hour in time required for the egg to reach the isthmus in birds with the longest and shortest intervals. If this 1-hour difference is accepted as being significant, it would seem that the lengthening of the interval is uniformly distributed throughout the oviduct. Twenty percent of the total variability of the interval is accounted for in the period required for ovulation and traversing the magnum by the egg and approximately that same percentage of the whole period of egg formation has elapsed when the egg reaches this point (posterior end of magnum). So, although the amount of the variation in length of interval accounted for in the magnum is small, it is as large as might be expected for a section of the oviduct in which the egg spends so little time. This relationship is indicated more clearly in figure 2. It may be questioned whether the 1-hour variation in time spent in magnum has any significance when the observations were made at 2-hour intervals.

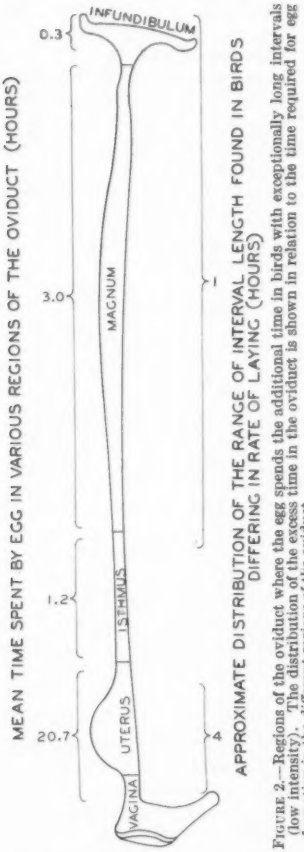

FIGURE 2.—Regions of the oviduct where the egg spends the additional time in birds with exceptionally long intervals (low intensity). The distribution of the excess time in the oviduct is shown in relation to the time required for egg formation in the different regions of the oviduct.

In an earlier study absolute or relative variations in time spent in the magnum, or time required for ovulation, could not be corre-

[6] WARREN, D. C., and SCOTT, H. M. See footnote 5, first reference.

lated with differences in mean interval length. If the variation found here is considered as significant it must be accounted for either as a delay in ovulation or a retardation of the rate of passage through the magnum in birds with longer intervals. Since so little variation was found in the rate of passage through the magnum and since there was considerable variability in time required for ovulation, the writers are inclined to designate the latter as the probable source of the lengthening of the interval in this region. The maximum variation in time between laying of the previous egg and the next ovulation in anesthetized birds was a little over 1 hour.

The data on time spent in the uterus by eggs having varying interval lengths are given in the second line of table 1. This period was measured by the time intervening between the egg reaching a plump stage and its being laid. The egg probably requires about 2 hours to become plump after entering the uterus, so this measurement is fairly accurate for estimating the period spent by the forming egg in the uterus. When the egg first enters the uterus the membranes are rather loose fitting and fluids then pass through them causing the egg to take on the plump state before any calcium deposition can be detected. The data in the third line of table 1 are the intervals between the time when the presence of shell material could first be detected and the laying of the egg. The shell formation can probably be detected in about 5 hours after the egg enters the uterus. The time of detection of either of these stages (plump or shell) may be used as a point from which to calculate the relative time spent in the uterus by eggs of different interval lengths.

It is shown that the period spent in the uterus, as measured by the time elapsing between the plump membranous stage (or the early shell formation stage) and laying, varies directly with the interval length. With each increase of an hour in interval length there is a corresponding, though not always equal, increase in hours spent in the uterus. For the 5 hours' difference in interval length, 3.6 hours are accounted for in the extremes from the plump stage to laying and 4.1 hours in the extremes between the first indication of shell formation and laying. In the former case 1.4 hours and in the latter 0.9 hour of the variation in interval are unaccounted for in the variations of time spent in the uterus. Since extremes of interval length are included perhaps one should not expect the means of time spent in the uterus to account for these extremes even though the uterus is the section of the oviduct where the variations in interval length occur. It can at least be stated that most of the variability in the absolute length of the interval may be accounted for by differences in time that the egg remains in the uterine region. It should be kept in mind that only those differences in interval are here considered which are found in birds having clutches of two or more eggs in size. Birds with 1 egg clutches were not included because of the impossibility of accurately calculating interval length. Also the long interval occurring between clutches is excluded.

It should be emphasized that normally approximately 82 percent of the interval is accounted for by the time the egg remains in the uterus. So if the difference in time of egg formation between long- and short-interval birds is uniformly distributed over the entire process of egg formation much of the difference would be accounted for

in the uterine stage. In accounting for the differences in interval length the time spent in the isthmus has not been discussed. The formation time in this region is so short and methods of locating the egg in this section of the oviduct so crude that it was not considered feasible to make comparisons in this region. It is also true that the differences encountered in interval length had all been accounted for in the magnum and uterus.

OVULATION DELAY AND CLUTCH

The foregoing study has accounted for much of the difference in interval length which is an important factor in rate of egg production. There remains a second factor which contributes to intensity or rate of laying. This is the length of the period between clutches. The occurrence of a break between clutches is due to a delay of a few hours in ovulation. For successive eggs in a clutch ovulation occurs practically immediately following the expulsion of the preceding egg. The length of the period interrupting the regular sequence of ovulation was measured by the period intervening between the detection of the presence in the oviduct of the first egg of the clutch and the laying of the last egg of the previous clutch. This period includes the time spent in the magnum by the first egg of the clutch as well as the period of delay in ovulation. These two factors cannot be segregated one from the other but it has already been shown that the former varies but little. Therefore, this interval may be used as a measurement of the variability in delay of ovulation. Since the length of the clutch is conditioned largely by the interval length, clutch size may be used for grouping the birds as to their interval length. They were placed in six arbitrary grades of increasing clutch size and the respective periods of delay of ovulation were 22.9, 22.0, 19.9, 19.0, 17.2, and 16.5 hours. It is evident that the birds with small clutches, long intervals, and low intensity, also have the longer delays in ovulation. Thus, the poor production of low-intensity birds is due not only to the longer period of egg formation but also to a longer delay in ovulation between clutches where such delays occur. From autopsies it was frequently noted that very low-rate birds showed evidence of ova having been released in the abdominal cavity. In such birds the rate of laying is probably not an accurate measure of rate of ovulation.

SUMMARY

It was shown that much of the difference in the absolute length of interval between eggs, which is the major factor in controlling rate of laying, is due to variations in time that the egg remains in the uterus. The time spent in the magnum (albumen-secreting section) plus the time between the previous laying and ovulation were only slightly variable and could account for only a small part of the absolute variation found in interval length. These conclusions were arrived at from data secured by tracing the forming egg while in the oviduct of living birds.

In low-intensity birds there was also a lengthening of the delay in ovulation which occurs in the case of the first egg of a clutch. Thus, low intensity of production is due to both a longer period for passage of the egg through the oviduct and to a longer pause between clutches.

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